HYDROLYSIS OF WHEY LACTOSE AND UTILIZATION OF THE PRODUCED SYRUP IN BAKING AND CONFECTIONERY

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DEDICATION

To my parents

Who lighten up my life with their support

To my wife

For here support and patience

To all my brothers and sisters

For supporting

To my ankle (Abu hothifah)

For his support

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Abbreviation	ABBREVIATION CAPTION
BOD	Biological Oxygen Demand
HTST	High Temperature Short Time
ONPG	O-Nitrophenyl –B-D- Galacto Pyranoside
ONP	O-NitroPhenol
LAU	Lactase Activity Unit
HPLC	High Performance Liquid Chromatography
H_2SO4	Sulfuric Acid
AOAC	American Association of Analytical Chemists
HCL	Hydrochloric Acid
PHEMA	Poly (2-Hydroxy Ethyl Methyl Acrylate)
AACC	American Association of Cereal Chemistry
CLHAWS	Concentrated Lactose Hydrolyzed Acid Whey Syrup

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ABSTRACT

Hydrolysis of lactose by β -galactosidase is one of the most promising biotechnological applications in food industry to produce low lactose dairy products. Labneh whey samples were collected from the dairy pilot plant at the University of Jordan, Faculty of Agriculture. β -Galactosidase enzyme from *Kluyveromyces lactis* with initial activity of 3000 LAU/ml was immobilized in a sodium alginate and used for whey hydrolysis in fluidized bed reactor. Optimization parameters of lactose hydrolysis were (12 and 24% v/v enzyme concentration), and (10 and 20 ml/min flow rate). The process reaction was done at a temperature of 37°C and 6.5 pH. Lactose hydrolysis of 81% and 85% were obtained after 6 hours of incubation for 12 and 24% v/v enzyme concentration at a flow rate of 10 ml/min.

The resultant sweet syrup was used as sugar replacer in three food products (French – type bread, Cherry cake and Kunafah syrup) at levels of 25, 50 and 75%. The bread dough made with 25% sugar replacement had better rheological properties with regards to stability, rate of absorption, and mechanical tolerance index. The sensory evaluation results showed that bread made with 25% sugar replacement awarded higher scores than control. Whereas, no significant differences were observed between Cherry cake and Kunafah, scores awarded for products made with 25% sugar replacement and control samples. The nutritional value of the newly developed products was improved with regards to protein quality.

1. INTRODUCTION

Whey is a by- product resulting from dairy industry especially cheese production. Both traditional and modern methods of cheese manufacture produce a huge amount of cheese whey (about 83% of the entire volume of milk used). Whey is classified to two types, sweet whey with a pH of about 6.02 to 6.58 and acidic whey with a pH of 3.57 to 4.34 (Alsaed and Ahmed, 2010). The majority of the globally produced whey is sweet whey produced as a by-product of cheese industry (Boutin, 2010). On the other hand, Labneh whey is only produced in some Mediterranean countries including Jordan.

Whey had been a major world–wide disposal and pollution problem for the dairy industry. Thus, nutritionally valuable food resource was wasted and added to the environmental pollution (Ponsano and Castro, 1995). Biological oxygen demand (BOD) for whey is considered to be high varying from 30.000 to 60.000 mg/L (Ponsano and Castro, 1995).

Boutin (2010) reported in a workshop held in Amman on 13 June 2010 by the U.S. Dairy Export Council, that no 1 ml of the American whey is wasted and there is a highly sophisticated whey industry in the country. He concluded that "I hope I have opened your eyes to new possibilities associated with whey where new products can be made and old products can be made better and lower in cost".

In Jordan where the majority of the produced whey is of the acidic type (a by-product of labneh production), there is still what can be called "the whey problem" i.e. disposing of acidic whey in the sewerage system and increasing the pollution level. For that reason, the Jordanian Ministry of Higher Education and Scientific Research sponsored in November 2008 a research project dealing with acidic whey and this study is part of that project.

The annual estimated amount of whey produced by the Jordanian dairy industry is about 40000 tons. More than three quarters of this quantity is acid whey and is produced from labneh, whereas the other is sweet whey that is produced from white cheese (Yousif *et al.*, 1998). It was noted that small amount of this whey (less than 1%) is used in the Jordanian food industry especially in beverages, whereas, the rest amount (99 %) of it is disposed in the sewage causing an environmental contamination problem due to its high BOD (Yousif *et al.*, 1997).

Hydrolysis of whey lactose by β- galactosidase enzyme (commonly known as lactase), may overcome some of the limitation in the utilization of lactose resulting from poor solubility, insufficient sweetness and the problem of lactose intolerance (Szczodrak, 1999). Also, the total solid of whey was recently used in the food production in different forms, as whey powder, concentrated whey, and totally or partially hydrolyzed lactose whey. Internationally there is a huge increase in the use of whey products in the food industry due to better understanding of the nutritional value of whey component (Mathur and Shahani, 1979).

Large number of papers could be cited from the literature related to sweet whey processing and utilization, compared to little or no work related to acid whey (Boutin, 2010).

The importance of this study might be clarified if we know that the annual Jordanian production of acidic whey (about 30000 tons) are being disposed in the sewerage system causing a serious pollution problem due to their high BOD levels. On the contrary, these huge amounts of acidic whey can be changed efficiently to a valuable high nutritious by-products by using different techniques as concentration, drying, permeation, lactose hydrolysis, … etc. At the same time, Jordanian imports of dried milk and other concentrated sugar syrups as glucose or fructose are estimated at

about (20,000 tons) which are used as sweeteners in confectionery and baking industry (DOS, 2009).

Therefore the objectives of this study are:

1.Conversion of acid whey lactose into glucose and galactose and production of sugar syrup by using immobilized β- galactosidase enzyme.

2. Utilization of acid whey hydrolysate in the local food processing, like bakery and confectionery industry, by improving the technological and sensorial characteristics of food items (such as increased solubility, greater sweetening power and lower caloric content of the products (glucose and galactose have greater sweetening power than does lactose))..

2. LITERATURE REVIEW

2.1 Whey definition

Whey is defined as the fluid obtained by separation of the coagulum from milk, cream, or defatted milk and from which a portion of the milk fat may have been removed (Blanc, 1977).

2.2 Composition of whey

Whey is classified into acid and sweet type. Acid whey is produced as a by-product of certain type of cheeses like, cottage, quark, and other fresh cheeses as well as labneh manufacture. It has a pH range of (4.2-4.8). Whereas sweet whey results from the manufacture of certain type of cheese like Cheddar and Mozzarella that principally use rennet type enzyme (Jelen, 1983; Jesus, 1984). The approximate compositions of sweet whey collected from six national dairy plants are (moisture: 92.78%, dry matter %: 7.22, Ash %: 0.48, NaCl %: 0.29, lactose %: 4.64, protein %: 0.96 and Fat %: 0.93) (Alsaed *et al* ., 2011).

Labneh (concentrated yoghurt), is a semisolid food derived from yoghurt by draining away part of its water and water-soluble compounds and has a cream/white color, a soft and smooth body, a good spreadability with little syneresis (Rasic, 1987).

2. 3 Liquid whey as a pollutant

Cheese whey represents an important environmental problem because of its high volume produced and its high organic matter content, exhibiting a (BOD) of 30000-50000 ppm (Marwaha and Kennedy 1988; Gonzalez, 1996). The production in the U.S.A of approximately 14.1 billion Kg of sweet type whey and more than 1.8 billion Kg of acid type whey with a collection of more than 1.0 billion Kg of solids per year has created a great disposed problem and gives great attention to the more efficient use of it. Each pound of whey has (BOD) of 23.000 mg (Gray and Amer, 1980; Jesus,

1984). Lactose is commercially produced from whey. Approximately 4.0 Kilograms (KG) of sweet whey are produced for each 0.5 Kg of cheese. In the case of cottage cheese manufacture, 2.7Kg of acid whey is recovered for every 0.5Kg of finished product (Thelwall, 1980; Jesus, 1984).

In 1970, and in a number of dairy factories, acid and sweet whey have been dumped into the sewerage system causing environmental problem. This problem becomes more serious due to the loss of protein and lactose, which could otherwise be recycled and reused efficiently. The awareness of economic implications of these considerations has encouraged the adoption of the following corrective measures:

- Pollution abatement programs by and for the cheese industry where it is a major contributor to the total industrial BOD load.

- Manufacture of protein concentrates and their utilization.

- Use of ultra filtration membrane for preparing food grade protein and reverse osmosis membrane for lactose concentration (Triveni, 1975).

2.4 Utilization of liquid whey

Whey contains nutrients which can potentially contribute to the vital needs of feeding world populations (Jelen, 1983). Whey proteins are composed of three major fractions: β Lacto globulin represents 66% of the total protein, alpha lacto albumin accounts for 22% of the total and immunoglobulin which represent 10% of total whey proteins. Small amounts of casein represents for the residual (Hambraeus, 1982; Jesus, 1984). In addition to high quality proteins, whey also provides calcium, phosphorus, sulfur and water-soluble vitamins. Excessive lactose and minerals contents can, however, limit the use of whey in feeding (Sienkiewicz and Riedel, 1990).

It was reported that, about 50%, of total world cheese-whey production is treated and used in many food products mostly in the european countries, about 45% used directly in liquid form, 30% in the form of powdered cheese whey, 15% of lactose and

delactosed by- products, and the rest as cheese whey-protein concentrates (Marwaha and Kennedy, 1988; Gonzalez, 1996).

2.5 Lactose

It is a milk sugar (4-B-D-galacto pyranosyl-D-glucopyranose) can be purified from cheese whey or permeate by crystallization. In percentage it represents the second high component of liquid whey after water percentage. It is a disaccharide composed of glucose and galactose. Lactose is less sweet than its sugar components, having a relative sweetness of 0.4 compared to 0.6 for galactose and 0.7 for glucose (Tamime and Robinson, 1985).

Lactose has been found in the milk of all Mammal species in a range of 2.0 to 8.5%; and it represents about 70% of whey solid contents (Thelwall, 1980).

2.5.1 Production and benefits of lactose

Current world cheese production is at least 9,000,000 tons per annum, resulting in whey contains about 4,000,000 tons of lactose. Current commercial utilization of lactose is about 200,000 tons, per year (Lander, 1981; Zadow, 1984). Most current uses of lactose in the food industry based on its unique physicochemical characteristics in comparison with other sugars. These characteristics include its ability to carry flavor and colors resulted in many food applications in product such as seasonings, and ranged of baked goods (Devero, 1973). The confectionery industry often uses lactose to obtain more desirable properties in the end products, relying on the property of lactose that changes crystallization characteristics of other sugars (Zadow, 1984).

Lactose can also react with proteins, peptides, and amino acids by the Millard reaction to form brown pigments (Guy, 1971). This potential browning agent property has been suggested as being of added value in microwave cooking, where low surface temperature limit browning as compared to that from conventional cooking methods (Tweedie *et al.*, 1978; Jesus, 1981). Furthermore, lactose is not fermented by baker's

yeast which means that any other functional properties conferred by addition of lactose are not lost during manufacture resulting in giving a unique characteristics to the baking industry (Zadow, 1984).

2.5.2 Some limitations of the use of lactose in the food industry

2.5.2.1 Low solubility.

A common problem associated with use of lactose in food industry is its low solubility. This is due to its crystallization in solution of a certain products. The presence of lactose crystals in dairy products forms defect when their size become large enough to produce sandy, or gritty texture; when they tend to settle and form a deposit, and also when their presence changes the physical character of a product or the ingredients thereof; or when they interfere with the use of the product. Any one of these effects may set in when the crystal length approaches or exceeds 30 μ. The products that mostly being affected are sweetened condensed milk, condensed and dried wheys, ice cream, plain condensed milk, dry milk and frozen milks. (Triveni, 1975). The only preventive method in the past has been to limit the size of crystals below 15 μ, also use of lactose prior to the condensing process to a point where crystallization is not a problem (Triveni, 1975; Kisza *et al.*, 1973).

Lactose has a different solubility (g/100g H_2O solution) at different temperatures (0°C : 11.9 , 25 °C: 21.6 , 64°C: 65,8 , 100: 157.6 °C) (Jesus,1984).

The newly emerging beta-galactosidase technology, offers solutions to the main three problems; lactose intolerance, pollution, and dairy product processing. In addition it offers new use of lactose as sweeteners for the food industry. More than 500 million pounds of lactose can be produced from some 10 billion pounds of whey each year in the United States alone, however the various outlets, such as the baking and pharmaceutical industries, have never in the past used more than 10% of preparing lactose. A better methods of preparing lactose by reverse osmosis process in addition to

a perfect utilization of a beta- galactosidase technology opened new avenues for the processing and use of lactose (Triveni, 1975).

2.5.2.2 Low sweetness

Lactose is relatively sweeter at higher concentrations compared to sucrose, although concentrations appear to have little effect on the concentration of sweetness of lactose compared to glucose or galactose (Poutanen *et al.*, 1978; Zadow, 1984). The relative sweetness of lactose at 25 °C is 15 compared to 100 and 70 for sucrose and glu-gal respectively at the same temperature (Jesus, 1984).

2.5.2.3 Lactose intolerance

Another significant limitation of using lactose in food industry is the inability of some individuals to digest and metabolize it normally. Lactose is the first carbohydrate available for developing mammals. In humans, lactose contributes 40% of the energy requirements consumed prior to weaning. To be utilized for energy, lactose must be hydrolyzed into its constituents (glucose and galactose) which can be readily absorbed (Thelwall, 1980).

The discovery that human infants and adults of certain ethnic groups suffer from a general and in some cases fatally acute, deficiency of lactase enzyme is a matter of great concern, and its implications were examined by various agencies, such as the U.S Office of child development and the protein advisory group of the United Nations Organization. Some 29 major case studies were published in the year 1972 alone to alarm the dairy and food technologist to the fact that lactose feeding is a serious problem in human nutrition (Triveni, 1975). Lactase is a specific beta-galactosidase and acts only on the break down of beta-1-4 glycosidic linkage of lactose and thus librating the monomeric sugars, glucose and galactose. Inability of breakdown of lactose leads to gastrointestinal problems like diarrhea, abdominal pain...etc (Kelly, 1984; Triveni, 1975). It has been estimated that about 70% of the world's population is lactose

intolerant (Bayless *et al.*, 1971). The implications of lactose intolerance in a sound nutritional policy and milk based food product development cannot be ignored (Hood, 1971; Kosikowski *et al.*, 1971). This does not mean that milk and milk products should be removed from our diets. The attempts to reduce lactose content of milk powders, concentrated cheese whey, and yoghurt, however, are definitely in the interest of human nutrition in general. The enzyme beta- galactosidase is being used on commercial scale to accomplish this objective (Wendorff *et al.*, 1971). Table (1) lists the tendency of world populations to lactose intolerance. For these reasons and limitations of lactose applications, the process of whey lactose hydrolysis is recommended. (Olling, 1972; Skala and Lamakova, 1971; Triveni, 1975)

Table 1: Tendency to lactose maldigestion

Area	**Tendency (%)**	**Area**	**Tendency (%)**
Western Europe		**Americas**	
Austria	15-20	North America (whites)	15
Balkans	55	North America (Hispanics)	53
Finland	17	North American (blacks)	80
France (north	17	South America	65 – 75
France (south)	< 70		
Germany	15	**Africa**	70 – 90
Great Britian	5-15	Bedouins	25
Italy	20-70	Touareg	13
Scandinavia	3-5		
Asia			
India (north)	30		
India (south)	70		
Central Asia	80		
East Asia	90-100		

Source: Asia Pacific Food Industry (June, 2000)

2.6 Beta-galactosidase

Lactase (β-galactosidase) is classified as a hydrolytic enzyme specifically as glycosidase for the hydrolysis of glycoside bonds producing glucose and galactose. It is found in the intestines of young mammals and in some plants, fungi, yeasts, and bacteria (Nijpels, 1981).

Enzymes of plants and animal origin have little commercial value but several microbial sources of β-galactosidase have a great technological interest (Finocchiaro *et al.,* 1980). However, β- galactosidase can be obtained commercially from different sources.

2.6.1 Sources of beta- galactosidase

2.6.1.1 Bacterial enzymes

The enzyme β-galactosidase can be produced by a large number of bacteria but *Streptococcus thermophilus* and *Bacillus stearothermophilus* are considered potential bacterial sources. The enzyme from *Escherichia coli* serves as a model for understanding the catalytic mechanism of β-galactosidase action. But, it is not considered suitable for use in foods due to the toxicity problems associated with the host coliform (German, 1997; Parmjit *et al.,* 2010). The first lactase derived from *Escherichia coli* was only used in analytical chemistry applications.

2.6.1.2 Fungal enzymes

a. *Aspergillus niger* lactase

This enzyme has various optimum conditions in comparison to yeast lactases. It has a highest activity at pH values (between 3.5 and 4.0). The maximum activity is observed at 60-65°C. A temperature of 50°C is recommended for thermal stability of the enzyme (Nijpels, 1982); it has a commercial value in the dairy and other food industries.

b. *Aspergillus oryzae* lactase

This enzyme has a wide range of pH-stability but it showed optimum activity at narrow range (between 4.5 and 5.0). It has a maximum activity at about 55°C. (Sprossler and Plainer, 1983; Victor, 1984).

2.6.1.3 Yeast enzymes

Yeast has been considered as an important source of β-galactosidase from industrial point of view. It has optimum activity at neutral pH. Therefore, yeast enzymes are considered suitable enzymes to be used for hydrolysis of lactose in milk and are widely accepted as safe for use in foods (Parmjit *et al.*, 2010).

a. *Kluyveromyces lactis* lactase

The optimum conditions for lactase activity located at a neutral pH range from 6.5 to 6.8, near the pH of fresh milk. The optimum temperature profile for activity is from 35 to 50°C. Although the enzyme activity is low at low temperature (5°C), the use of this enzyme has been considered for the treatment of milk during overnight cold storage period (Nijpels, 1982; Victor, 1984).

Whole milk and sweet whey can be treated directly using this enzyme; whereas the acid whey should be adjusted to the proper pH required for enzyme action prior to hydrolysis. This enzyme is inhibited slightly by sodium and ammonium ions, so that, potassium hydroxide is considered a suitable neutralizing agent (Nijpels, 1981).

b. *Kluyveromyces fragilis* lactase

This commercial enzyme preparation is obtained by submerges fermentation (Anonymous, 1981). It has similar conditions for hydrolysis as reported for the *K. lactis* enzyme. The optimum activity can be reached at pH 7. It has a moderate stability to temperature. As in *K. lactis*, its activity is greater in the presence of K^+ ions rather than Na^+ ions (Dahlgvist *et al.* 1977).

2.7 Immobilization of beta-galactosidase

The immobilized enzyme is defined "as the enzyme physically confined or localized in a certain defined region of space with retention of its catalytic activity, which can be used repeatedly and continuously." (Chibata, 1978). Immobilization of β-galactosidase can affect enzymes properties; e.g. temperature, pH, kinetic parameters, stability...etc (Rossi *et al.*, 1999). If a proper technique is applied, immobilization process can improve properties of β-galactosidase such as enzyme stability at high or low pH and temperatures (Zhou and Chen, 2001; Juan, 2009).

A variety of methods have been used to immobilize β-galactosidase from different sources with different degrees of success. The choice of suitable enzymatic preparations based on its properties and the purposes of its application. Although the enzyme β-galactosidase has numerous applications in the food and dairy industries, but the moderate stability of enzyme is one of the limitations that restrict general implementation of biocatalysts at industrial scale (Parmjit *et al.*, 2010).

The majority of immobilized systems of β-galactosidase have been developed for the purpose of lactose hydrolysis. Yeast β-galactosidase is normally used for hydrolysis of lactose in milk and sweet whey, whereas fungal β-galactosidase is more suited for acidic whey hydrolysis. Fungal β-galactosidase is more sensitive to product inhibition, mainly by galactose compared to yeast enzymes (Boon *et al.*, 2000; Parmjit *et al.*, 2010).

2.7.1 Immobilization methods

β - galactosidase enzyme can be immobilized using several methods, including entrapment, adsorption, covalent binding, and cross linking. The selection of immobilization methods depends on many factors like, enzyme, reaction, reactor, matrix ...etc (Tanaka and Kawamoto, 1999).

2.7.1.1 Physical adsorption

Physical adsorption is considered as the simplest method of immobilization in which an enzyme is immobilized onto a water-soluble carrier and the biocatalysts are held on the surface of the carriers by physical forces (van der waals forces). Frequently, additional forces are involved in the interaction between carrier and bio catalysts basically hydrophobic interactions, hydrogen bridges, and heteropolar (Ionic) bonds (Hartmeier, 1986; Parmjit *et al.*, 2010). The disadvantage of this technique is the relative weakness of the adsorptive binding forces. A variety of materials has been used, alumina, stainless steel, glass … etc.

2.7.1.2 Entrapment

This method is based on the localization of an enzyme within the lattice of a polymer matrix or membrane entrapment (including micro- capsulation). The major advantage of this method is the simplicity by which spherical particles can be obtained by dripping a polymer-cell suspension into a medium containing positively charged ions or through thermal polymerization (Hartmeier, 1986), also, beads formed particularly from alginate are transparent and generally mechanically stable.

The major limitation of this technique is the possible leakage during repeated use due to the small molecular size compared to the cells. However improvements can be made using suitable linking procedure for β-galactosidase immobilization, the lattice method is the most widely used technique over other four types of entrapment; microcapsule, liposome, membrane, and reverse micelle (Tanaka and Kawamoto, 1999; Juan, 2009). Alginate polymer (naturally occurring polysaccharide) is the most common matrix used for entrapment of β-galactosidase enzyme.

2.7.1.3 Covalent binding

Covalent binding is the retention of enzymes on support surface by covalent bond formation. Enzyme molecules bind to support material by certain functional groups such as amino, carboxyl, hydroxyl, which are not essential to the catalytic activity (Parmjit *et al.*, 2010). The major disadvantage of this method is low activity and high cost and need technical experts (Tanaka and Kawamoto, 1999).

2.7.1.4 Cross-linking

This method is utilized as bi-or multifunctional compounds, which serve as the reagent for intermolecular cross-linking of the biocatalyst. It is usually used in combination with other immobilization method, mainly with adsorption and entrapment. (Grosova *et al.*, 2008).

2.8 Lactose hydrolysis techniques

Several processes for the conversion of lactose into glucose and galactose present at a commercial, near commercial or significant pilot plant development stages. The hydrolysis of lactose may be catalyzed either by β-galactosidase enzyme or by acid (Victor, 1984). The enzymatic techniques of whey lactose hydrolysis includes the free enzyme system, immobilized enzyme system, and acid hydrolysis technique.

2.8.1 Free enzyme hydrolysis

The simplest enzymatic method of lactose hydrolysis in whey is the direct addition of β-galactosidase to the whey in a batch process. This method has been successful for the lactose hydrolysis in milk (Dahlgvist *et al* ., 1977). The required amount of enzyme is determined by many factors; the activity of the preparation used, the desired degree of hydrolysis, the temperature, pH, time of the reaction and the minerals content of the whey. The hydrolysis process is terminated by inactivation of the enzyme by conventional HTST (high temperature short time) pasteurization. Neutral enzyme has been used in the treatment of whole sweet whey (Burguess and Shaw, 1983;

Victor, 1984). In order to optimize whey lactose hydrolysis a temperature of 35- 40°C and reaction time longer than 6 hours were recommended when using neutral enzyme. Although some neutral β-galactosidase preparations were active at temperature as low as -9°C (Baer and Loewenstein, 1979; Victor, 1984), this would not be suited for the hydrolysis of whey. Since at low temperature, the enzyme productivity was too low to be economical, there was a possibility for hydrolysis of concentrated whey at low temperatures. However; product inhibition could be a significant problem with a concentrated substrate (Giec and Kosikowski, 1983).

The main disadvantage of this process is that after hydrolysis, the enzyme remains in the product and is therefore lost. A process was developed to improve the utilization of the enzyme by recovering the enzyme by ultra filtration techniques (Miller and Brand, 1980).

2.8.2 Immobilized enzyme system.

A number of immobilized enzyme systems have been developed for whey processing applications. A patented hydrolysis technology developed by Corning glass is the best-known system. This system utilized an *A.niger* β-galactosidase immobilized on controlled pore size silica beads, and has been reported to be used commercially to produce lactose hydrolyzed syrups, in a joint venture of Corning and an American supermarket chain (Anonymous, 1981; Victor, 1984). In England, the milk marketing board and Corning had set up a joint company to produce and market a sweetener obtained from whey (Anonymous, 1982). The New Zealand Dairy Board was involved with Corning similar enterprise. (Anonymous, 1982). Whey lactose hydrolysis was examined using fluidized bed reactor. The result showed that the activity was dropped rapidly over the first 30 hours because of absorption of the solid matter but then stabilized to a slower decline. Cost was estimated at US $ 170/t for a half-life of the enzyme of 60 days. Annual return of investment was calculated at 44% in commercial

operation (Julkowski, 1981; Zadow, 1984). Coughlin, *et al.* (1978) examined hydrolysis of acid whey using a fluidized bed containing lactase from *A.niger* on a porous aluminum carrier, a slow but steady reduction of activity was observed on treatment of whey, probably due to absorption of solids onto reactive sites. Furthermore, the immobilized *K. Lactis* β-galactosidase on to CPC-silica (Silanizated and activated with glutaraldehyde) and agarose displayed 90% conversion in packed bed mini reactor (Giacomini *et al.*, 1998). *K. fraglis* β-galactosidase immobilized on silanized porous glass modified by glutraldehyde binding retained more than 90% of its activity (Szczodrak, 2000; Parmjit *et al.*, 2010).

Aspergillus oryza β-galactosidase enzyme was immobilized on reactive carrier of Glycidyl methacrylate (GMA) – Styrene (ST. Satisfied results were obtained concerning pH stability, thermal stability and operational stability compared to free enzymes (Sun and Xu, 2010).

Aspergillus oryza β-galactosidase enzyme was adsorbed using simple adsorption mechanism with native Zinc oxide (Zno) and Zinc oxide nanoparticles (Zno-NP); the hydrolysis rate for milk lactose in batch process after 9h was 54%, 63%, and 71%. While whey lactose was hydrolyzed at 61%, 68% and 81% under similar experimental conditions (Husain *et al.*, 2011).

B-galactosidase enzyme was immobilized by adsorption on a mixed-matrix membrane containing Zirconium dioxide. The Michaelis constants (Km) value increased almost eight times compared to free enzyme, the maximum enzyme velocity (V max)remained almost constant (Peter *et al.*, 2011).

2.9 Mechanism of lactose hydrolysis

Enzymatic hydrolysis of the glycosidic bond of lactose takes place via general acid catalysis that requires two critical residues; i.e. a proton donor and a nucleophile/base. The mechanism of lactose hydrolysis was first described by

Wallenfels and Malhotra (1960), where lactose from *E. coli* was used. The proposed mechanism of reaction based on the action of cystine and histidine residues, in which they acted as proton donor and nucleophile site, respectively, during the enzymatic hydrolysis procedure (Quinn and Xiao, 2001).

It has become clear recently, that the β-galactosidase from a variety of microbial origins has two glutamic acid residues (Glu482 and Glu551) as the proton donor and the nucleophile /base at the same time in the enzymatic reaction. The reaction mechanism is shown in (Fig.1). The first step is the formation of enzyme-galactosyl complex. As a result, glucose is simultaneously liberated. In the second step, enzyme-galactosyl complex is transferred to an acceptor containing a hydroxyl group. In a diluted lactose solution, water rather than other sugars such as glucose, lactose can be more competitive to be an acceptor, therefore galactose is formed and released from the active site (Quinn and Xiao, 2001). On the other hand, in a high lactose content solution, lactose molecule has more chances to act as the acceptor, binding with the enzyme-galactose complex to form oligosaccharides (Figure 1 and 2).

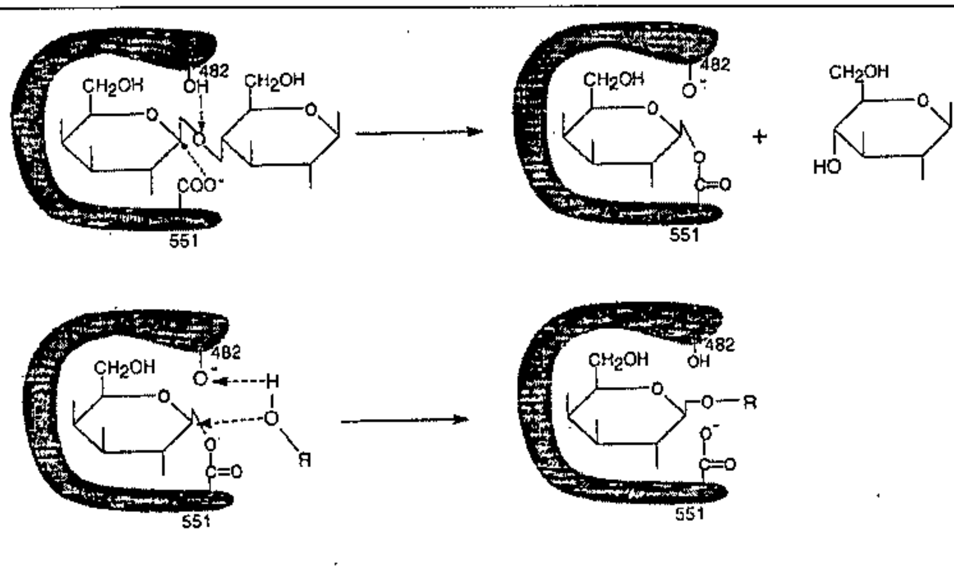


Figure 1 : Mechanism of lactose hydrolysis by beta-galactosidase enzyme
Source: (Quinn and Xiao,2001)

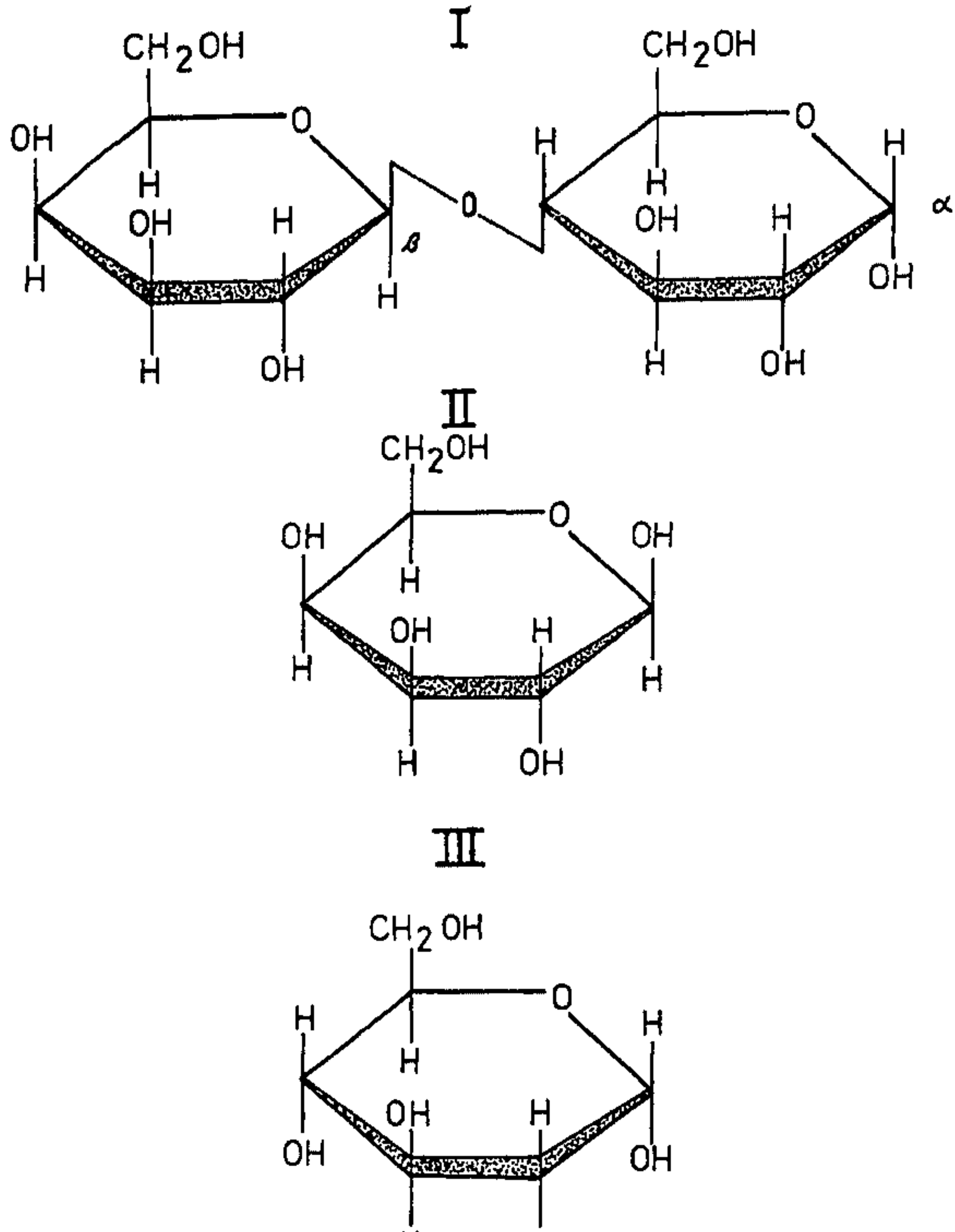


Figure 2: Lactose structure (glucose and galactose)

Source: (Zadow,1984)

2.10 Application of lactose hydrolysis with immobilized beta-galactosidase

The immobilized β-galactosidase from *A.oryzae* in a packed reactor displayed 80% of lactose hydrolysis in whey (Plainer *et al.*, 1984), whereas the immobilized β-galactosidase from *Saccharomyces fragilis* resulted in a hydrolytic rate of 50% within 3 hours in recycling packed bed reactor (Gonzalez and Suarez Doval, 1994). Cross-linked enzyme aggregates of β-galactosidase were found to be effective in lactose hydrolysis yielding 78% monosaccharide in 12 hours of incubation (D'Souza *et al.*, 1985). Lactose hydrolysis of 75% was obtained within 5-6 hours using immobilized fungal β-galactosidase into polyvinyl alcohol gel (Batsalova *et al.*, 1987).

Calcium alginate entrapped β-galactosidase was used for the hydrolysis of lactose in milk and whey in batch process as well as in continuous packed bed column. It was also observed that entrapped cross-linked concanavalin (A) β-galactosidase was more efficient in the hydrolysis of lactose present in milk (77%) and whey (88%) in both processes compared to the soluble β-galactosidase (Batsalova *et al.*, 1987). Furthermore, the immobilized *K. Lactis* β-galactosidase on to CPC-silica (Silanizated and activated with glutaraldehyde) and agarose displayed 90% conversion in packed bed mini reactor (Giacomini, *et al.*, 1998).

In a study conducted by Jurado *et al.*, (2002) it was shown that the optimum conditions for *Kluyveromyces fragilis* enzyme hydrolysis were achieved at pH 6.6, and at 37°C temperature. Chien, *et al.*, (2002) studied the optimization of enzymatic process for manufacturing low-lactose milk containing oligosaccharides, they reported that the optimum conditions for hydrolysis was 2.5% on initial lactose concentration, at 50°C for 3.5 hours.

In another study, it was shown that the percentage of lactose conversion was optimized to be 37°C and pH 7 for 24 hours using immobilized β-galactosidase from *Kluyveromyces marxinus saccharomyces* on different supports (Di Serio *et al.*, 2003).

In addition to that, *K.lactis* β-galactosidase immobilized on cotton fabric using glutaroldehyde as the cross-linking reagent was used for hydrolysis of lactose in whole milk and 95% of lactose conversion had been observed after 2h of batch operation (El-Gindy, 2003).

The lactozyme (*K. fragilis*) β-galactosidase was immobilized on cellulose-beads and obtained 90% of whey lactose hydrolysis and 60% conversion in 5 hours, and the immobilized enzymes could be reused three times without any changes in the performance of fluidized bed reactors (Roy and Gupta, 2003).

Enrique and Amelia (2006) found that the optimum conditions for lactose hydrolysis using packed-bed reactor were at 37°C temperature, 12% v/v enzyme concentration, and pH at 6.85 using 3% Na-alginate in the immobilization. Elcin, *et al.* (2009) carried out a modeling study on hydrolysis of whey lactose and stability of β-galactosidase, they found that the optimum conditions for lactose hydrolysis were at 50g/L lactose concentration, ImL/L enzyme concentration, and pH 6.5 at 37°C.

The use of immobilized β- D- galactosidase (Ec.3.2.1.23) for the production of lactose-hydrolyzed milk and whey hydrolysis to obtain glucose and galactose is one of the most important applications of enzyme in food processing since there is value in the production of lactose-hydrolyzed milk and whey (Reeba *et al.*, 2010).

The β-galactosidase enzyme of fungal sources immobilized on hydro gels was used for whey hydrolysis and 70-75% hydrolysis was achieved within 7 hours (Kozhucharova *et al.*, 1990; Parmjit *et al.*, 2010).

A new strategy to immobilize β-galactosidase enzyme was developed by using polymeric supports having Schiff bases which prepared from (aminomethyl)

Polystyrene and 2- Phenlyindole-3-carboxaldehyde by condensation. The influence of temperature, pH, reusability and storage capacity on the free and immobilized enzyme was investigated. The results indicated that Schiff bases is most suitable for the immobilization of β-galactosidase due to their strong storage capacity and reusability (Aynaci *et al.*, 2011)

2.11 Sweeteners production from whey

The hydrolysis of lactose in milk and other dairy products into its components (glucose and galactose) increases sweetness intensity of the product (Pomeranz, 1964; Kisza *et al.*, 1973; Engel, 1973). This is an important development, espically in the lack of food sweeteners. The preparation of food syrups from acid whey after recovering the protein is a good step forward (Wierzbicki and Kosikowski, 1972). However, the syrups prepared in this manner cannot be used on part per part basis as substitute for corn syrup and other commonly used sweetening agents because of the expected low sweetness power of the syrup. Although the intensity of sweetness is not linearly correlatable with the concentration of different sugars, the sweetness of the syrup may be expected to be three to four times higher even for unconcentrated (lactose hydrolyzed) whey; further increases in the sweetness power of the syrup can be obtained depending on the degree of concentration. A further improvement in the sweetness power of the syrup had been suggested with regards to isomerizing glucose to fructose by using another immobilized glucose isomerase reactor on the line which uses beta-galactosidase reactor effluent as feed for isomerization reactor system (Weetall *et al.*, 1974; Triveni, 1975). This could be obtained after a final effluent of six to seven times sweeter than the original whey, further increase in sweetness can be achieved by concentrating the effluent syrup. Glucose isomerase is already being used as an industrial catalyst coupled with glucoamylase (Olson and Richardson, 1974). Its technology is probably one of the most well-utilized food enzymes.

2.11 Use of hydrolyzed syrups

Hydrolyzed whey syrups could be used as sweeteners in dairy and confectionary product (Coton and Wingard, 1976). Applications of lactose hydrolyzed whey to ice cream, water ice, caramels, and syrups were discussed by (Holsinger, 1978).

De La Gueriviere (1978) reported that hydrolyzed lactose can be used instead of sucrose in the manufacture of bread, cakes, and biscuits, Lactose hydrolyzed milk is suitable for the preparation of concentrated milk products, Ice cream, water ice, baby foods, Cheddar cheese, yoghurt and caramels (Woychik and Holsinger, 1977; Zadow, 1984; Holsinger , 1978). The production of flavored drinks from fluid whey is a relatively simple process which avoids expensive steps such as drying, condensing (Szakaly *et al.*, 1978).

A sweet syrup prepared by acid hydrolysis of Cheddar cheese whey permeate was used for sucrose replacement in manufacturing syrup of commercially canned peaches and pears. It was noted that After 3 months storage browning did not appear in all tested syrups. A further storage for 15 months showed slight differences in sweetness intensity of organoleptically tested syrups (Tweedie and Macbean, 1978).

Guht, (1978) reported that Fruit-Salad quary products containing partially or wholly hydrolyzed lactose had longer storage life than control samples. For more effective utilization of cheese whey in cake and pastry, whey was treated by lactase enzyme and 64% of the total lactose was hydrolyzed to glucose and galactose, thereby, increasing the sweetness of thc whey and further improvement of its flavor. Hence the sugar requirement for cake preparation was also reduced (Keshavarz and Nakai, 1984).

Several alternatives have been suggested for the utilization of whey permeate (Horton, 1982). One of these is to hydrolyze the lactose in permeate and use it as a sweetener to replace a traditional sucrose or corn syrups. It was reported that,

satisfactory ice creams were made with hydrolyzed permeate syrups by substituting for up to 10% of the sucrose (Holsinger and Roberts, 1976). Moreover, Rexroat and Bradley (1986) indicated that it was possible to replace sucrose in soft - service ice milks at the 100% level with hydrolyzed permeates syrups without lowering over all quality.

Lactose and hydrolyzed lactose products have long been recognized as useful ingredients in bakery products (Pomeranze *et al.*, 1962). It also may help increase the specific volume of a loaf and yield good texture and grain (Vetter, 1984). Holmes and Lopez, (1977) indicated that, the replacement of 20 to 30% of sucrose with lactose was desirable in products that require low sweetness, limited solubility, strength and hardness of crystals, whereas the addition of hydrolyzed lactose products was desirable for improving the appearance, flavor and aroma, volume and shelf life of some bakery goods.

It was also reported that the replacement of sucrose with a high fructose corn syrup at 15 and 25% replacement level in white layer cake product resulted in a good texture and darker color of both crust and crumb (Saussele *et al* ., 1976). More in depth, 100% replacement of non-dry milk with heat treated whey in cake mix and dough mix, was beneficial to bakers as it would reduce the ingredient costs and to cheese manufacture as it would alleviate a waste disposal problem (Keshavarz and Nakai, 1984). Furthermore, studies regarding bakery and fancy bakery products, using of hydrolyzed whey concentrates resulted in products with good properties like crust, cream and volume (Bednarski, *et al.* 1979).

The utilization of hydrolyzed whey in the baking industry could replace whole milk, skimmed and whey powders. The utilization of whey as a fermentation substrate has been studied extensively (Kosikowski, 1979). Salmon (1981) examined the use of

demineralized - lactose hydrolyzed whey in products such as milk, toffees, milk sweets, spreads, fondants, candy bars, cake, and biscuits. He found that the use of hydrolyzed lactose did not cause a harmful effect in confectionary and flour-based industries, generally, 50% demineralization was adequate. A nutritional beverage can be developed and considered very promising area for the successful utilization of hydrolyzed whey. The preparation of an orange flavors shelf-stable athletic-type beverage made from lactose hydrolyses and deproteinated whey base was reported by Crippen and Jeon, (1984).

A comparison was made between the addition of lactose hydrolyzed products into the normal formulation and addition of lactase to the dough during bread making. Both samples compared to the control showed higher loaf volume and higher crumbs compressibility (Zadow, 1984).

The lower sweetness of glucose/galactose is useful in the processing of foods in which a high osmotic pressure is required to reduce bacteriological activity but in which excessive sweetness is undesirable. In jams, in particular, it may be used to yield a product in which the fruit flavor is not masked as severely by excessive sweetness as when sucrose was used (Zadow, 1984).

Ogunrinola, *et al.*, (1988) incorporated hydrolyzed whey permeate syrup (HWSP) as substitution for sucrose in a typical white pan bread formulation. They found that there were significant differences in the yeast fermentation rate, proof time, and bread quality among dough formulated with sucrose and (HWSP). Yousif, *et al.* (1998) reported that the external and internal characteristics of the French type bread were improved by the addition of whey solids.

It was reported that the protein content of breads gradually increased among the supplemented bread with oleic-rich sunflower seed at supplementation ratio of (8, 12

and 16% flour basis) (Biljana and Bojana, 2008). Furthermore , it was demonstrated that, when fortified with defatted maize germ at (5 ,10, 15 , 20, or 25% flour basis) in cookie formulation, crude protein, crude fat, crude fiber and ash content were significantly improved ($p \leq .05$) with all level of replacement. In another study, it was found that substitution of wheat flour with resistant starch type (iii) in butter cake at levels of (0, 5, 10, 15 and 20%) caused a decrease in the levels of ash, protein and fat content with increased levels of substitution. This might be explained on the basis of, that resistance starch has lower protein, fat and ash content than wheat flour (Pongjanta *et al* ., 2008).

Whey protein is considered to be of a high quality type, it is composed of three major fractions: beta lacto albumin which represents 66% of the total protein, alpha lacto albumin accounts for 22% of the total and Immunoglobulin which represent 10% of total whey proteins. Whereas small amounts of casein represents for the residual (Hambraeus, 1982). The increased level of protein content in the treated samples (CLHW) compared to the control is expected to improve the nutritional value of these products. Since whey proteins are rich in lysine and tryptophan which are the first and second limiting amino acids in wheat flour. These whey proteins can increase the nutritional value of the wheat protein by improving the amino acids balance (Singleton and Robertson, 1966).

3. MATERIALS AND METHODS

3.1 Sample collection and preparations

Acid labneh whey samples were collected from the dairy pilot plant at the University of Jordan/Faculty of Agriculture from the labneh production line. Potassium hydroxide (Sigma-Aldrich- St. Louis. Mo USA) was used to neutralize the acidity to the desired pH value (Rajakala and Karthigai Selvi, 2006; Wierzbicki and Kosikowski, 1972), and pH meter (Hanna instruments Hi 8519-Italy) was used to monitor the pH of the treated whey. Whey sample was sterilized by membrane filtration (cellulose nitrate with 0.2 mm pore size and 47 mm diameter, Microfiltration system, U.S.A). Then the sample was introduced into the fluidized bed reactor for lactose hydrolysis (Figure 3).

Acid whey

⇩

Addition of potassium hydroxide

⇩

Enzyme hydrolysis

⇩

Sweet whey

⇩

Concentration

⇩

Sugar replacer in French bread, Cake, and Kunafah

⇩

Rheological properties of bread dough

⇩

Product characterization (Chemical analysis, Sugar analaysis, and Bread storage study)

Figure 3: Steps included in the processing of acid whey lactose hydrolysis

3.2 Enzyme immobilization

β -galactosidase enzyme (3. 2.1.2.3) from *Kluyveromyces lactis* (Sigma-Aldrich-St. Louis. Mo USA) was used in the experiment. The enzyme was mixed with an aqueous sodium alginate solution at 3% (w/v) (Sigma-Aldrich St. Louis. Mo USA). Beads were prepared by dropping the solution using 10 ml sterile syringe into 2% w/v calcium chloride solution (Frutarom-LTD, UK). The alginate polymer it self is anionic (i.e. negatively charged) whereas the calcium is cationic (i.e. positively charged). Thus, the replacement was occurred according to the following equation:

$$2\ Na\ (alginate) + Ca^{++} \Rightarrow Ca\ (alginate)_2 + 2\ Na^{+}$$

All operations were carried out under aseptic conditions (Sienkiewicz and Riedel, 1990)

3.3 Bioreactor design

The bioreactor used was of Fluidized bed type reactor. It was composed of peristaltic pump (Manostat-Varistaltic pump-advanced model-USA), reactor column (8.3 cm × 20 cm), glass reservoir of 2L capacity fitted with inlet and outlet connected pump. The temperature was controlled using water bath (Memmert model- Germany). Three - way valve was used for sampling under aseptic conditions reaction conditions described above (Figure 4 and Figure 5)

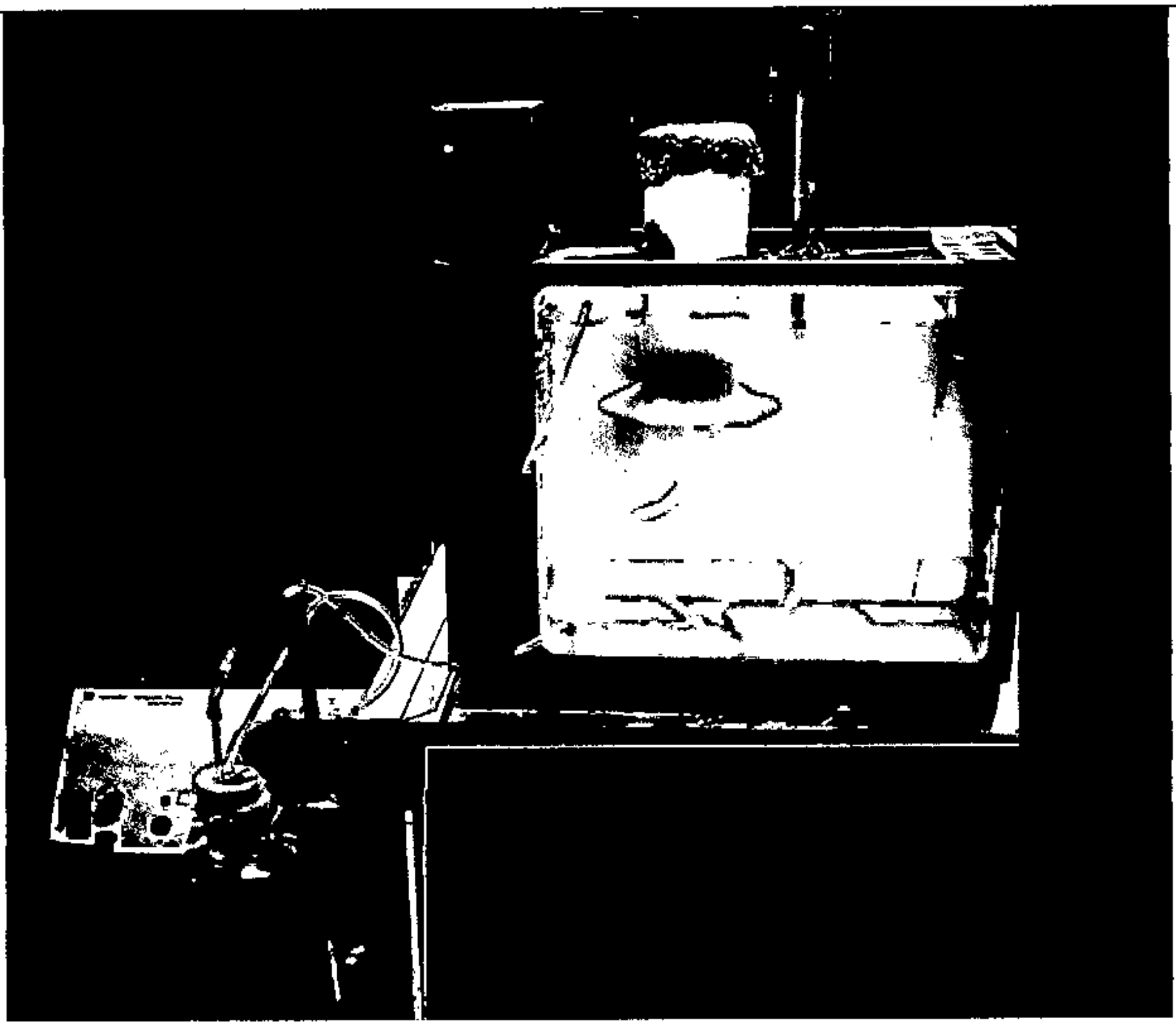


Figure 4: Fluidized bed reactor (designed in the laboratory)

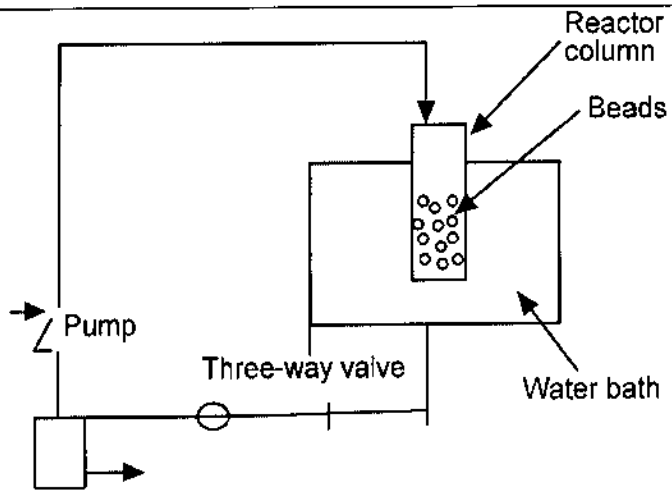


Figure 5: Fluidized bed reactor (diagram)

3.4 Determination of enzyme activity

Lactase activity on O-nitrophenyl β - galactosidase (Fluka-Biochemika, USA) (ONPG) was determined by adding 25 μl of enzyme (equal 1mg protein) solution to 980 μl of substrate solution (860 μl with 50 mM potassium phosphate buffer at pH 6.5 and 120 μl with 25 mM ONPG diluted into 50 mM potassium phosphate buffer at pH 6.5). The added enzyme and substrate were incubated at 37°C for 5 min, then after the reaction was stopped by adding 1ml of 0.5 M sodium carbonate. The absorbance of the nitrophenolate was measured at 420 nm using spectrophotometer (Aleco-SL150 UV-Spectrophotometer). One unit of ONPG activity is defined as 1 μ mol of ONP released per minute under reaction conditions. A standard curve was established by dissolving various concentrations of O-nitrophneol (Fluka-Biochemika, USA) in the assay solution. (Rajakala and Karthigai Selvi, 2006; Novalin *et al* ., 2005).

3.5 Optimization process for lactose hydrolysis

Optimization of different factors for acid whey lactose hydrolysis was carried out on stepwise procedure to attain maximum lactose hydrolysis. The initial volume of the sample was (1L). The reactor column was packed with 250 ml of Na- alginate beads. Two flow rates (10 and 20 ml/min) and two enzyme concentrations (12 and 24 v/v) were used in this optimization. Table (2) shows physiochemical properties of the used enzyme. Enzyme hydrolysis was done at fixed temperature of 37°C and, 6.5 pH (Table 3). Whey samples were flowed from the packed column under the effect of gravity to the reservoir and was continuously recirculated using peristaltic pump to flow through the column for the determined incubation time of 8 hours. The degree of hydrolysis, defined as the percentage of lactose molecules cleaved out at the total amount of molecules initially present, can be measured by the determination of the amount of lactose utilized or by amount of glucose produced.

The percent of lactose hydrolysis (H %) was measured using the following equation:

$$H\% = \frac{\text{Initial lactose concentration (ppm)} - \text{lactose concentration at time interval (ppm)}}{\text{Initial lactose concentration (ppm)}}$$

The residence time was measured using the following equation:

Residence time = packed column volume (ml) / flow rate (ml/min) = ml/flow rate (Szczodrak, 2000).

Table 2: Physiochemical properties of used enzyme as supplied by manufacturing company

Property	Lactosyme 3000 L
Physical state	Liquid
Density	1.2
Initial activity (LAU/ml)	3000
Protein concentration (mg/ml)	40

Table 3: Experimental parameters for acid whey lactose hydrolysis

Factors	Variables
Temperature (° C)	37°C *
Lactose concentration (%)	2-4
Enzyme concentration (v/v)	12 and 24 v/v of Na-alginate
Ph	6.5 *
Flow rate (ml/min)	10 and 20

* as recommended by manufacturing company

3.6 Sugar determination of the hydrolyzed syrup

For determination of initial and residual lactose concentration, samples were taken from the reactor every one hour. The glucose and lactose amount in these samples were determined using HPLC method. The HPLC (Knauer Model, Germany) composed of pumping system, (Smart pump 1000), software program (Autochro-3000 version 2.0.4). The HPLC column used in the analysis was (Unisol Amide 4.6 x 190mm column-Agela company) and refractive index detector (RI) (Acmc 900 Younglin-South Korea), with a mobile phase of acetonitrile (Sigma-Aldrich- St. Louis. Mo USA): water (75:25) at flow rate of 1 ml /min was used. The experiment was carried out in triplicate

and the averages were taken (Dimitris *et al.*, 2005; Manazanres *et al.*, 1993). Figures (6, 7, 8 and 9) show standard curves of the determined sugars which reflect the highly accuracy of the carried tests and results (Appendix 2).

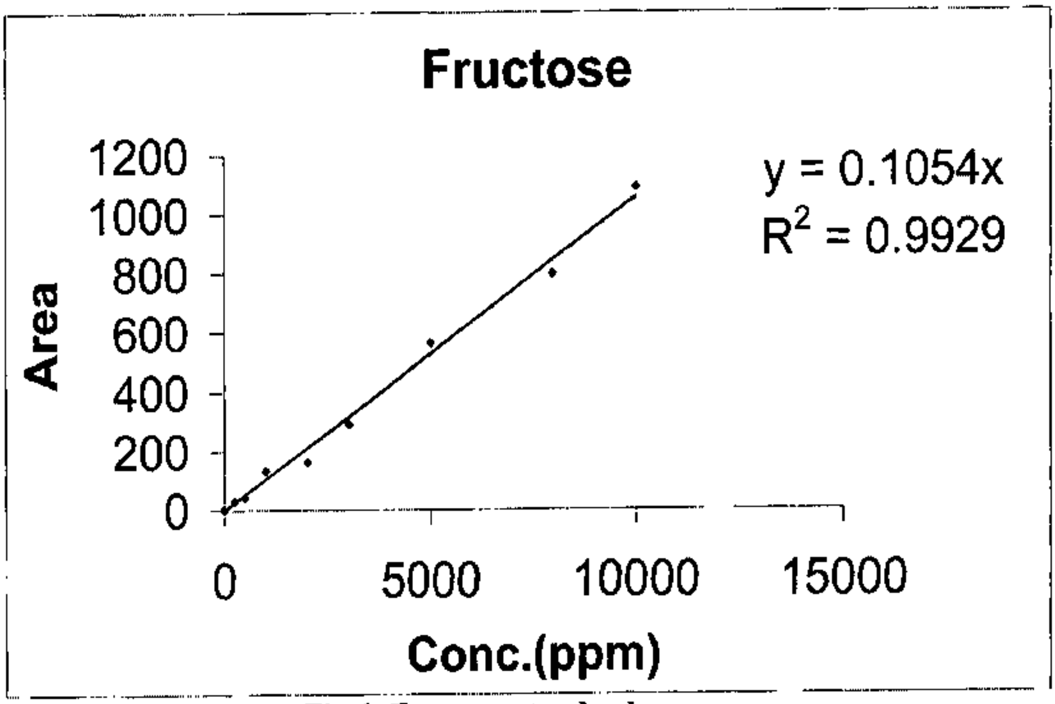


Fig 6: Fructose standard curve

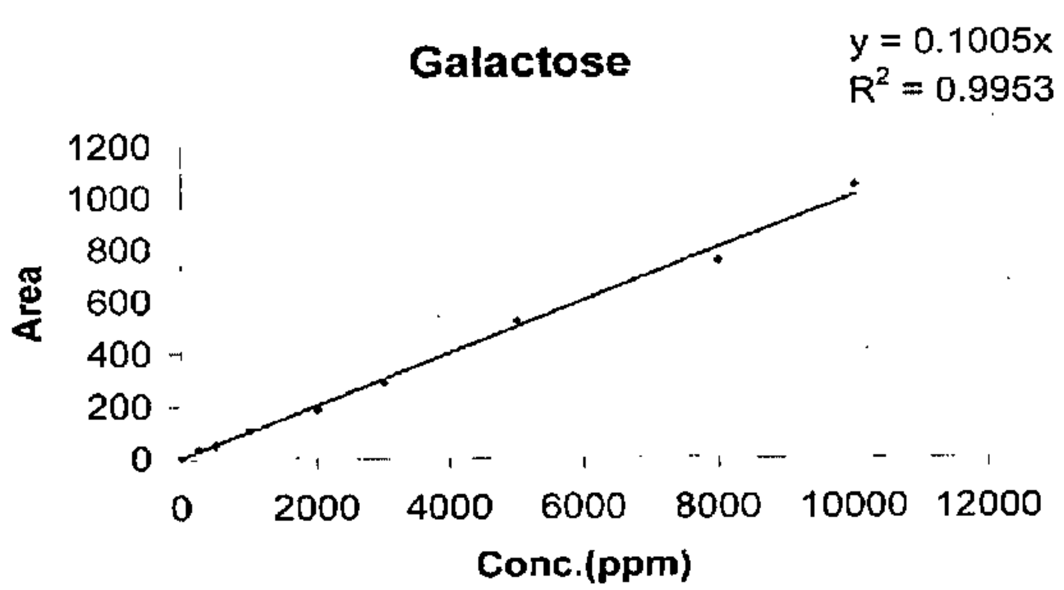


Fig 7 :Galactose standard curve

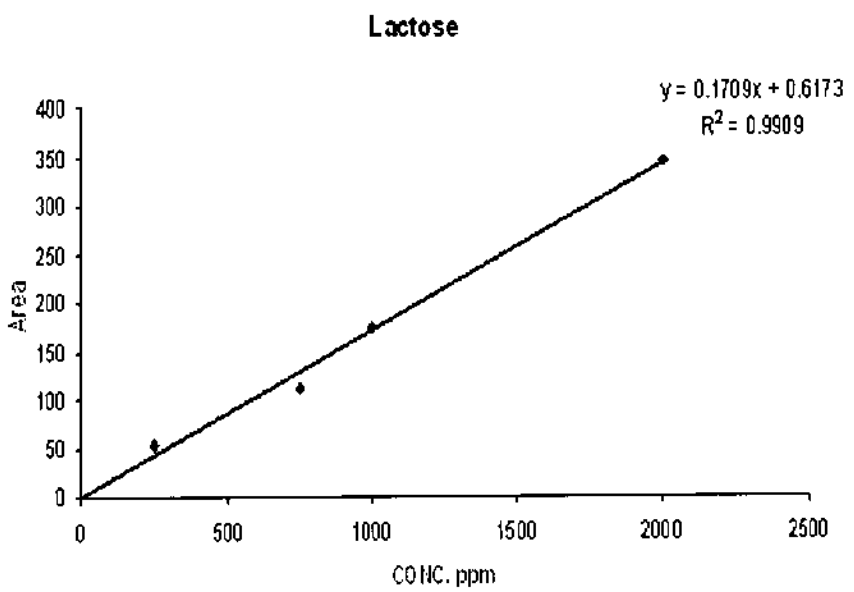


Fig 8:lactose standards curve

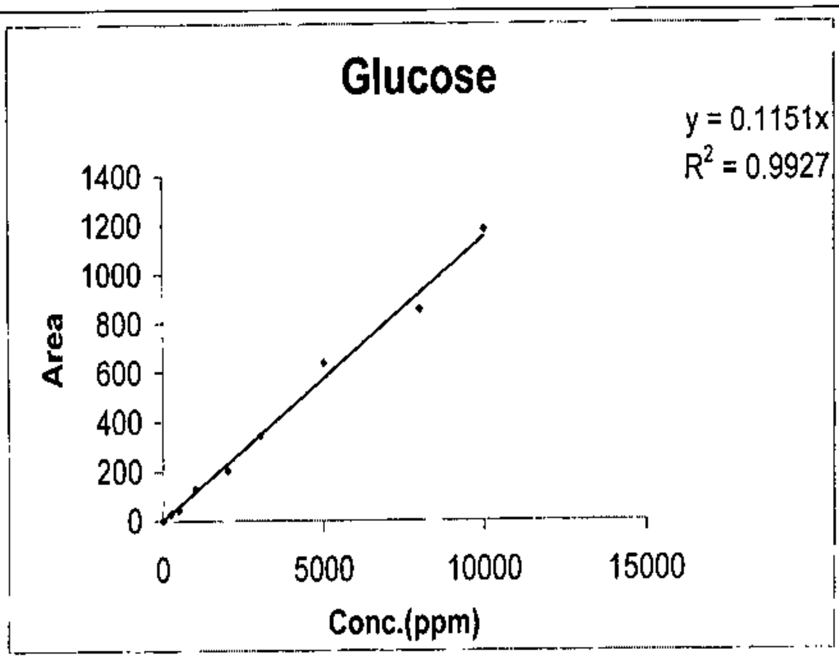


Fig 9: Glucose standard curve

3.7 Hydrolyzed lactose (sugar syrup) concentration

The hydrolyzed lactose in acid whey samples (20 Kg) with initial total soluble solids of 6% were concentrated using vacuum evaporator supplied with mixer (Appendix 1); (manufactured by Zhejiang Wenxiong Machine Valve Co., China; its model is ZN50) with an evaporating ability of 20 kg/h, heat area of 0.4 m^2, condensation area 1.2 m^2, and cooling area 0.25 m^2. The concentration process was carried out at temperature 70°C, vacuum and pressure at 0.03, mpa, time or duration needed for concentration of (20 Kg) was 45 minutes), the total solids achieved (20, and 40 %) (Appendix 1).

3.8 Rheological properties of the bread dough

The rheological properties of the bread dough were evaluated using Farinograph according to the AACC method (2000). Flour sample of (300 g) on 14% moisture basis was weighed and placed into the corresponding farinograph mixing bowel. Water from burette was added to the flour and mixed to form dough. As the dough is mixed, the farinograph records a curve on graph paper. The amount of water added (absorption) affects the position of the curve on the graph paper. Less water increases dough consistency and moves the curve upwards. The curve is centered on the 500 – BU (brabender unit) line ±20 by adding the appropriate amount of water and was run until the curve leaves the 500-BU line. All experiments were done in a duplicate and the results were averaged

3.9 Test baking

The experiments regarding the replacing of sugars by the concentrated sugar syrup in one bakery item (French -type bread), and two confectionery products (Kunafah and Cherry cake) were carried out in the University of Jordan (JU) restaurant. The methods and recipes for preparation of the 3 mentioned items by the (JU) restaurant were followed (Appendix 6).

3.9.1 French-type bread

The control dough was prepared using the straight dough bulk fermentation process. All ingredients i.e flour (2 kg), water (about 1000 ml), salt (30g), yeast (50g), improver (30g) and sugar (200g). Three experimental bread doughs were prepared having 25, 50 and 75% actual sugar replacement. The dough's were mixed until they had achieved proper development. The dough's were first proofed for 5-10 minutes at 35°C, then divided into 80g pieces, moulded, panned and finally proofed at 45° C and 85% relative humidity until a suitable height had been attained. The loaves were baked at 230°C for 15 minutes using a rotating oven and then allowed to cool in the pans for 10 minutes before depanning.

3.9.2 Cherry cake

The control dough was prepared using the straight dough preparation. All ingredients i.e water (1 L), Sugar (150 g), powdered cream (350g), gelatin (50 g), canned cherry (10 g) as flavoring agent. Three experimental cake doughs were prepared having 25, 50 and 75% sugar replacement by sugar syrup. The dough's were mixed until they had achieved proper development. Then the product was kept in the refrigerator to achieve the desired firmness and consistency.

3.9.3 Kunafah syrup

The control syrup was prepared using (600 ml) water and (300 g) sugar. The syrup was heated until boiled and becomes semi viscous, three experimental sugar syrup were prepared having 25, 50 and 75% sugar replacement by hydrolyzed lactose syrup. Then the prepared syrups were added to the previously prepared Kunafah; consisting of (500g) ready Kunafah dough, (100g) ghee, and (1kg) cheese. The dough's were mixed until they achieved a proper development. Then the previous ingredients were added and heated for proper period of time. The prepared sugar syrups (control, 25, 50 and 75%) were added to end product and served hot.

3.10 Chemical analysis of product samples

3.10.1 Protein determination

Total nitrogen content of bread and Cherry cake were determined using Kjeldahl method as outlined in the AOAC method (1995). The sample (around 0.2g) was firstly digested by H_2SO4 (98% w/w) using (Digester 2020, FOSS) at temperature of 380°C with the addition of the digestive mixture composition to break down the organic matter and reduce nitrogenous compounds to ammonium salts. Ammonia was liberated by boiling with sodium hydroxide 50% (w/v) aqueous solution and steam into boric acid to form ammonium borate. Ammonia was then titrated with 0.1N HCL, screened methyl red was used as indicator solution. Protein % was calculated using the following equation:

Protein percentage = Nitrogen % * specific factor (5.7 for the bread and 6.25 for other samples).

3.10.2 Ash content

Ash content was determined according to the AOAC method (2000). Two to three g of product sample were weighed in pre- weight porcelain crucible, the crucible and its content were placed into Muffle Furnace (Thermolyne, 600 Furnace) at 600°C for 3-4 hr. The crucible was cooled in desiccator and weigh. Ash % was determined using the following equation: Ash % = (M2-M1/M3) * 100 where,

M1: crucible weight

M2: crucible weight plus residue weight,

M3: sample weight.

3.10.3 Water activity

Water activity was measured using Novasina instrument (Axier Ltd); the method given in the manual of the instrument was followed; the temperature was calibrated to reach 25°C, then switching on the instrument for 10 to 15 minutes. The sample was put in plastic plate then placed in the specified chamber. The instrument was left until the reading of the water activity appears on the screen of the instrument (AOAC, 1995).

3.11 Sugar extraction and determination (for the developed products)

The sugar contents of the developed products were extracted using a method described by (Langemeier and Rogers, 1995), with slight modifications. Five g of each sample (bread ,Cherry cake and Kunafah syrup) was placed in 100ml volumetric flask with 70% ethanol. The sample was homogenized in a homogenizer (Ultra turex mixture, Type 25, Germany) for 5 min at 15000rpm and then sonicated for 15 min at 45°C. The sample was then filtered using (Whatmon filter no.1). The filtrate was microfilterd using 0.45 µm nylon –type membrane. Then the sample was ready for HPLC analysis. Using (NH2-R-P-Macherey-Angel-Germany) with 79:21 aceotonitril: water at flow rate of 1ml/min.

3.12 Sensory evaluation.

The produced products (French-type bread, Cherry cake and Kunafah) were evaluated by a panel of assessors (12 semi-trained subjects) from the Department of Nutrition and Food Technology at the Faculty of Agriculture of the University of Jordan. Bread samples were evaluated for the sensory attributes on a 9 point hedonic scale, with 9 indicating "dislike extremely" and 1 "like extremely". Testing was conducted in one session in a well- lighted and odor-free environment with specific instructions for sample evaluators. Kunafah and Cherry cake were evaluated for taste and aroma. The sensory evaluation for the three products were done twice in duration of 1 week. (Ogunrinola *et al.*, 1988; Yousif *et al.*, 1991) (Appendix 5).

3.13 Bread storage study

The bread samples was wrapped in polyethylene bags, sealed and stored for 5 days at 20°C. Bread samples were checked every day (Yousif *et al.*, 1998).

3.14 Statistical analysis

The analysis of variance (ANOVA) was done using mixed procedure (Proc Mixed) to perform a Repeated Measure Analysis Model whereby time was considered as the repeated factor. Differences between treatment means were tested using protected Least Significant Difference (LSD) test at $p<0.05$. All statistical analyses were performed using the SAS software, version 9 (SAS Institute 2002). (Appendix 4).

4. RESULTS AND DISSCUSIONS

4.1 Determination of enzyme activity

One unit of ONPG activity is defined as 1 μ mol of ONP released per minute under the reaction conditions. A standard curve was established by dissolving various concentrations of O-nitrophneol in the assay solution. (Figure 10). The absorbance of ONP at 420 nm was 0.240. By using the mentioned equation in the curve, the concentration of ONP was (0.063 Mm) within 5 min of incubation. Thus it was indicated that one unit of (LAU) of this enzyme for ONPG was 12.54 μ mol per min/mg protein. This result is in agreement with that obtained by (Morisi *et al.*, 1972). They reported that the activity of yeast β – galactosidase enzyme on ONPG substrate was 12.3-μ mol per mg of protein compared to 9.0 and 7.0 μ mol of substrate hydrolyzed per mg of protein with lactose and milk, respectively.

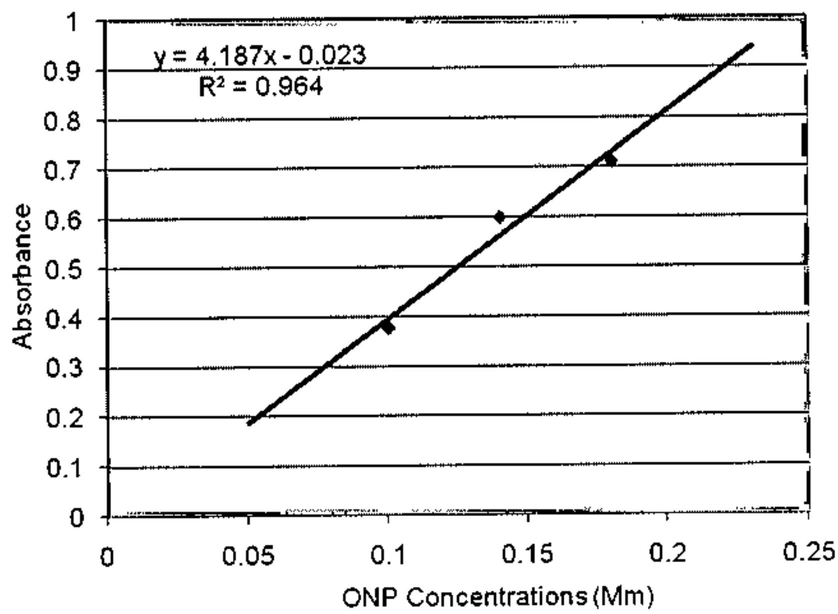


Figure 10 : ONP standard curve

4.2 Optimization of lactose hydrolysis process

Results obtained indicated that the amount of lactose (3.6 %) was relatively high and feasible to be converted into glucose and galactose for producing sweeteners to replace sucrose in different products. This result is comparable to the results obtained by Coton and Wingard, (1976). The relatively high level of lactose could be attributed to the use of a special starter culture (YCX 16) with low activity in lactose hydrolysis into lactic acid during labneh production, (Chris Hansen Company).

The maximum whey lactose hydrolysis was obtained at 24% v/v enzyme concentration after 6 hours of incubation which reached about 85% (Table 5 and Figure 12).

Enzyme concentration of 12 % v/v showed lactose hydrolysis value of 76.5% and 81.7% for the rate of 20 and 10 ml/min, respectively (Table 4 and Figure 11). These results are in agreement with those reported by Toshiba and Qayyum, (2009a) who found 89% of lactose hydrolysis in whey after 3 hours, while in milk, the hydrolytic level of lactose reached 79 % after 4 hours with the use of immobilized β -galactosidase in Ca-alginate – starch beads using batch process. Also, Toshiba and Qayyum (2009b) reported the value of 86% of lactose hydrolysis in whey using packed bead column of Ca-alginate beads with an entrapped β-galactosidase enzyme during 4 hours of incubation. Other researchers reported 87% lactose hydrolysis in milk using packed bed reactor with an immobilized yeast cell in Na-alginate beads after 7 hours of incubation (Reeba *et al.*, 2010).

The increase in lactose hydrolysis with an increase in enzyme concentration (24% v/v) compared to (12% v/v) could be due to the increase of enzyme availability which increased the rate of hydrolysis (Reeba *et al.*, 2010). Similar conclusions were reached by Szczodrak (2000), where 71% of lactose hydrolysis was obtained at enzyme concentration of 1 U/g lactose compared to 86% at 5 U/g lactose.

The rate of lactose hydrolysis was measured at two different flow rates (10 and 20 ml/mint) in the packed bed column. Maximum lactose hydrolysis (85%) was obtained at 10 ml/min flow rate rather than at 20 ml/min. This result could be attributed to the residence time (time by which the process fluid passed through the reactor) (Quinn and Xiao, 2001). For the first flow rate, the residence time was 25ml/flow rate, whereas for the second flow rate, the residence time was 12.5ml/flow rate. This indicated that, as the residence time increased by decreasing the flow rate, the availability of enzyme to substrate was increased and thus the rate of hydrolysis was also increased. These results are in agreement with those reported by Genari *et al.* (2003), where 80 % of lactose hydrolysis was achieved at 30 ml/min flow rate using continuous system with recirculation. Also, Quinn and Xiao (2001) reported 90 % of lactose hydrolysis at flow rate of 7ml/min using fluidized reactor with immobilized β – galactosidase on graphite slabs. Furthermore, Szczodrak (2000) found that the lactose hydrolysis at flow rate of 0.7ml/min was 87.3% compared to 82.6% hydrolysis at 2 ml/min flow rate when used immobilized lactase on silanized porous glass modified by glutaraldehyde binding.

It is clear from the Figure (11) and Figure (12) that the rate of hydrolysis was increased gradually up to 6 hours of incubation reaching maximum rate of lactose hydrolysis of 85%, and then the rate of hydrolysis remained nearly constant. The stability of lactose hydrolysis values following maximum hydrolysis might be ascribed to several reasons, such as an increase in the inhibiting concentration of glucose, or exhaustion of an activator, such as lactose, or even some proteolysis of the enzyme (Genari *et al.*, 2003). It was reported that a high milk lactose hydrolysis was obtained using a continuous milk (plug flow) system with recirculation compared to the system without recirculation (Genari *et al.*, 2003). Moreover, this satisfied the major objective

of this research in which cost reduction of lactose conversion was a major target of our research. Immobilized β –galactosidase was used with 2- days intervals and reused (10 times) in a total period of 20 days. The results showed that, there were no considerable activity changes within the test period (Sibel and Ozlem, 1999). The immobilized enzyme was preserving its original activity even after the 10 times of use within 20 days.

Szczodrak (2000) reported that more than 90% of initial enzyme activity after covalent binding of the enzyme on porous silanised glass without any noticeable decrease in enzyme activity during 5 repeated batch conversion. Similar results of good operational stability of immobilized enzyme was obtained with β – galactosidase immobilized in /on poly (2-hydroxyethyl meth acrylate) (PHEMA) membranes by two different methods; adsorption on Cibacron F3GA derivatised PHEMA membranes and entrapment in the bulk of the PHEMA membranes. After 15 repeated batch conversions the retained activity of adsorbed and entrapped enzymes was 80 and 95%, respectively (Baran *et al.*, 1997). These studies support the theory of the immobilized enzyme (cross- linkage) in enhancing enzyme stability and activity after long using in lactose hydrolysis. It was reported that microbial contamination was associated in the immobilized enzyme system. Therefore, using immobilized enzyme on large-scale continuous processing of milk necessitates the introduction of intermittent sanitation steps, which include the use of regular sanitation with basic detergent (Novalin *et al.*, 2005). Recently, two types of β –galactosidase: thermo stable and cold- active enzymes have increasing interest in industrial lactose hydrolysis process to avoid microbial contamination. The thermo stable β – galactosidase have the ability to retain their activity at high temperature for prolonged period and could be used in the industrial processing of dairy products simultaneously with heat treatment to sterilize the product

(Wolosowska and Synowiecki, 2004). The cold- active β –galactosidase provide treatment of milk and dairy foods below 5°C so that the taste and nutritional values remain unchanged (Fernandes *et al.*, 2002).

Table 4 : Lactose hydrolysis (%) at 12% v/v enzyme concentration at different flow rates

Incubation time (hour)	%lactose hydrolysis	
	10 ml/min	20 ml/min
1	40.20A*	21.8B
2	47.53A	28.90B
3	55.46A	36.50A
4	75.13A	48.40B
5	77.86A	56.9B
6	81.7A	74.9B
7	81.56A	76.76A
8	81.53A	76.50B

*Means in each row followed by the same letter are not significantly different at 95 % confidence.

Table 5: Lactose hydrolysis (%) at 24% v/v enzyme concentration at different flow rates

Incubation time (hour)	%lactose hydrolysis	
	10 ml/min	20 ml/min
1	47.76A	26.36B
2	56.66A	38.26B
3	62.60A	51.50B
4	79.70A	77.80B
5	83.33A	81.16B
6	85.03A	83.16B
7	85.09A	83.96B
8	84.86A	82.63B

*Means in each row followed by the same letter are not significantly different at 95 % confidence.

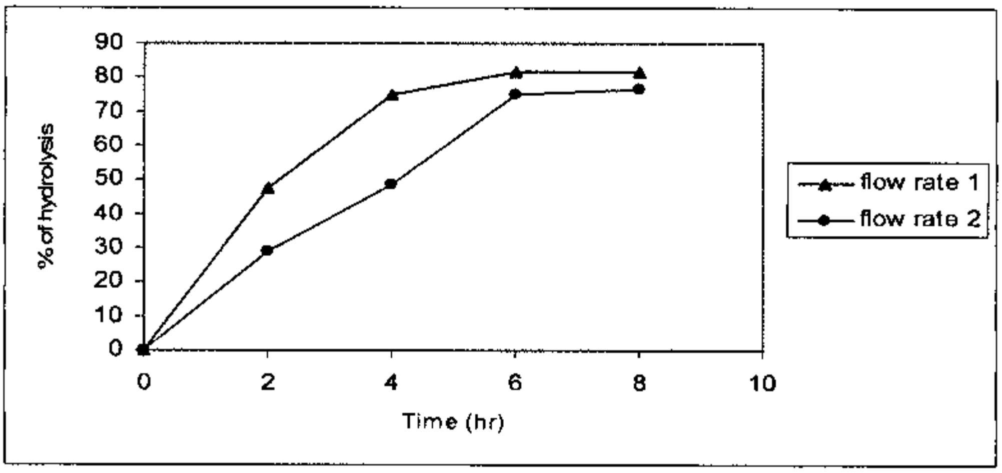


Fig 11: Whey lactose hydrolysis at 12%v/v with two flow rates (10 ml/min) and (20 ml/min)

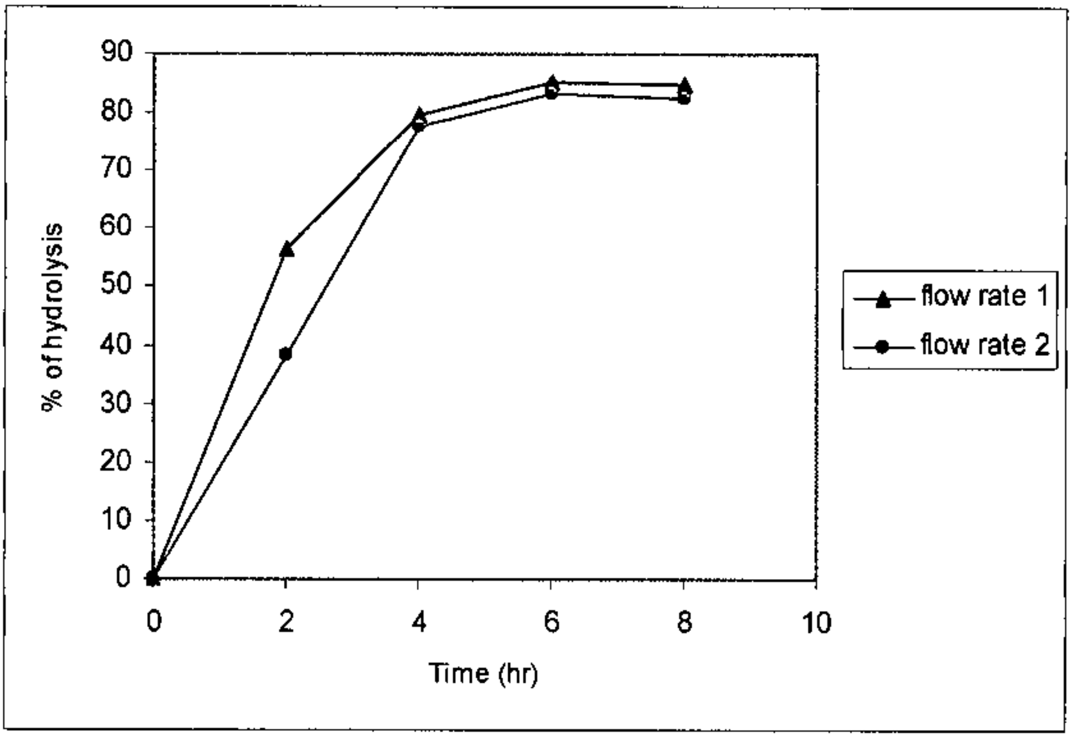


Fig 12: Whey lactose hydrolysis at 24%v/v with two flow rates (10 ml/min) and (20 ml/min).

4.3 Farinograph results

Table (6) and appendix (3) show the results of farinograph analysis. The results indicated that, the addition of concentrated lactose hydrolyzed acid whey syrup (CLHAWS) increased significantly the rate of water absorption ($p \leq .05$), although this increase was relatively slight. It ranged from 60% for control sample to 66% for the 75% sugar replacement level. These changes may not be due to sugar only because whey contains proteins, salts and other soluble components (yousif *et al.*, 1998). However, these results agree with those reported by Yousif *et al.* (1997) ; Thompson and Baker, (1983). They reported that the addition of total solids from whey increased the rate of water absorption.

Addition of low percentage of sugar syrup (25% replacement level) positively improved dough stability. Our results are in agreement with those reported by Bilgin *et al.* (2006) who found that, the addition of whey and buttermilk significantly increased dough stability, due to the role of physiochemical properties of whey and buttermilk which contain different protein sources, milk fat and lactose. On the other hand, increasing the added percentage of sugar syrup produced similar conclusion which was obtained by Abdalla et *al.* (2011) who reported negative effect on dough stability. These results may be due to the different reactions under the conditions of dough preparation. Sulphydryl/disulfide group may play as an important factor in controlling the properties of dough.

It is clear from the rheological properties (Table 6) that, the mechanical tolerance index (MTI) significantly increased ($p \leq .05$) in the three levels of sugar replacement. (MTI) measure the degree of softening during dough mixing, the higher the (MTI) the weaker the flour. (MTI) reached its maximum value (60 BU) at 50% replacement level, whereas it was (50 BU) at 25% and (21 BU) at 75% replacement

level. These results indicated that, the addition of hydrolyzed syrup did not affect significantly (p>.05) the dough development time. These results agreed with those reported by Yousif *et al.* (1998).

Table 6: Farinograph characteristics of bread dough

Treatment	Absorption (%)	Dough development time (min)	Stability (Min)	Mechanical tolerance indication
25% replacement	61.0000 BC	2.2000 A	13.7500 A	50.3000 B
50% replacement	63.2500 AB	1.6000 B	3.3750 B	60.0500 A
75% replacement	65.5000 A	2.1000 A	1.3750 D	21.5000 C
Control	60.4000 C	2.0500 AB	2.3000 C	20.0000 D

*Means in each column followed by the same letter are not significantly different at 95 % confidence

4.4 Chemical analysis results

Protein, sugars and ash percentages as well as water activity content were presented in Table (7). The results showed that, the addition of (CLHAWS) was slightly but not significantly increased the protein content of both bread and Cherry cake products compared to control. It was clear from (Table 7) that protein content was increased as a function of increased level of added whey syrup. Protein content of the bread was ranged from (9.37%) in the control to (10.13%) in the bread with 75% sugar replacement level. On the other hand, they ranged from (10.5%) for the control to (11.50%) for the 75% sugar replacement level in cake products. Jooyandeh *et al.* (2009) found that, supplementation of flour by fermented whey protein concentrate and whey permeate at 25, 50, 75 and 100% levels, increased the bread contents of protein, fat, and ash. The values of these components increased with increasing levels of substitution.

Regarding the ash content, it was significantly ($p \leq .05$) increased as the level of sugar replacement level increased from 25% to 75%. It ranged from (1.32%) for the control sample to (2.35 %) for the 75% replacement level for bread. For Cherry cake, the values ranged from (0.50%) for the control to (2.12%) for the75% replacement level. these results are in agreement with those reported by Bilgin *et al.* (2006) who found that, the replacement of water with pasteurized whey and butter milk increased the macro minerals concentration, particularly the calcium and potassium levels ($p \leq .05$) of the bread samples compared to the control. The ash content of the bread samples reflects its contents of minerals. Such results were expected since whey contains different amounts of minerals like (Ca, P, Mg, k and Fe).

Due to the low water activity of bread (Table 7) the 75% replacement level had a better stability or shelf life than other treatment, water activity was (0.39) compared to (0.49) for the control. However, the water activity of samples with 25%, 50% replacement and the control sample was less than (o.6) indicating a good stability or storability and shelf life for these products (Yousif *et al.*, 1990). This may indicate the possibility of longer storage of bread at ambient temperature (20 ± 5°C) without deterioration. However Cherry cake water activity was significantly ($p \leq .05$) decreased as the sugar replacement level increased.

Water activity can be defined as the amount of water available for chemical reactions and microbial growth. The reported decrease in the water content as a function of increased level of sugar replacement might be due to the available total solids like, protein, sugars, salts and minerals. Proteins become denaturated during baking process, as a result, the functional groups of denaturated whey protein becomes active and hydrated by free water. The same trends can be also followed to explain the effect of sugars on water activity. Sugars have a hydrophilic nature so, it can bind the free water and reduce its quantity, and thus, the water activity. These results, however, agree with other earlier findings on water activity in baking products. It was found that, sugars are used in the food industry to lower water activity. They decrease the water activity as the concentration increase; however, the rate of changed of water activity per unit of concentration is different for different sugars (Sloan and Labuza, 1968, 1975). In addition to that, it was reported that, small solutes such as sugars and salts lower water activity in baking products by increasing starch gelatinization temperature (Sloan and Labuza 1975; Ghiasi *et al.*, 1982).

Dramatically physical, chemical and biological changes took place during bread making, such as evaporation of water, formation of porous structure, volume expansion, protein denaturation, starch gelatinization , crust formation ...etc. All of these changes might affect the chemical and physical analysis of the bread samples (Arapita and Datta , 2008).

Table 7: Chemical analysis of developed products

treatment / composition	25% replacement	50% replacement	75% replacement	Control	LSD values
French-bread					
Protein %	8.4100 BC*	9.6500 AB	10.7900 A	6.9900 C	1.45373
Ash %	1.3650 C	1.8000 B	2.6050 A	1.3250 C	0.12836
Water activity	0.4550 B	0.4160 C	0.3965 C	0.4920 A	0.0208
Cherry cake					
Protein %	4.3000 BC	4.9900 AB	5.4500A	3.8150 C	0.895
Ash %	0.6750 C	1.2050 B	2.1200 A	0.5000 D	0.15521
Water activity	0.6910 B	0.6180 C	0.5940 D	0.7310 A	0.01778

*Means in each row followed by the same letter are not significantly different at 95 % confidence

4.5 Sugar analysis results

The results of sugar analysis of the developed three products (French – type bread, Cherry cake, and Kunafah syrup in which 50% sugar syrup was chosen as an intermediate added added percentage) were presented in Table (8) and Figure (13) Significant differences ($p \leq .05$) were observed between (sucrose, glucose, galactose, fructose and lactose) content. Control bread contained (2.15%) sucrose, whereas in the 50% replacement level it was (1.95%). Since the French-type bread dough contains about 8% sucrose, so a marked decreased is clear in sucrose content of the bread. These results were in agreement with other findings (Yousif and Alghzawi, 1990; Blenford, 1979; Calxito and Canellas, 1982) who reported that roasting of carob powder caused some reduction in sugar content due to the Millard reaction and carmalization. Nearly all sugars in the hydrolyzed syrup are invert sugars and therefore it is expected to play a major role in the browning process. During baking above (160°C) sugar undergoes a series of complex browning reactions (Czernohorsky and Hooker, 2010). The products of such reactions form the brown crust of many baked goods, these reactions are known as Millard reactions, and are essentially amino acid-catalysed caramelisation reactions in which a sugar aldehyde or ketone is converted to an unsaturated aldehyde or ketone (Czernohorsky and Hooker, 2010).. Furthermore, it was found that, lactose is responsible for the improved crust color and flavor development in bakery goods when a dairy ingredient is used, as it combines with protein in Millard reaction (Singleton and Robertson, 1966). The formation of crust and browning during baking appears to be the primary contribution to the formation of bread flavor. The browning is mainly the result of Millard type browning reaction rather than of carmalization. Crust browning occurs when the temperature is greater than 110°C and it showed an experimental correlation with weight loss during baking and with oven temperature (Mondal and Datta, 2008).

Another chemical reaction that might affect the sugar content was fermentation process, where sucrose is considered the main ingredient to produce carbon dioxide and moisture. Fermentation is the process by which yeasts act on the sugars and starches to produce carbon dioxide and alcohol. This process is the fundamental step to making bread dough (Mondal and Datta, 2008). It was demonstrated that, lactose was not hydrolyzed by Bakers yeast during normal fermentation steps in bread making, and was thus available to undergo the Millard reaction which contributes to color development in bread crust (Guy, 1978).

The same trends can also be noted for sucrose level in the 50% replacement level treatment. It was clear from Table (8) that, galactose was not detected in the control sample compared to (0.95%) in the 50% replacement level. Presence of galactose in the 50% replacement level treatment was due to the hydrolysis process conducted on lactose which produce glucose and galactose that comes from added syrup. Regarding lactose, small amount of this sugar (0.3%) was detected in the 50% replacement level treatment compared to (0.0%) in control sample. This residual amount of lactose originated from the added syrup, because the hydrolysis rate of lactose was optimized at 85% after 6 hour. These results were in agreement with the previous results reported about lactose hydrolysis; 87% of lactose hydrolysis was reported in milk using packed bed reactor with an immobilized yeast cell in Na-alginate beads after 7 hours of incubation (Reeba *et al.*, 2010). In addition to that, last century Shah and Nickerson (1978) found that, 100% lactose hydrolysis was not necessary, since maximum sweetness was achieved with 70-90% hydrolysis rate, due to the synergistic sweetening effect of lactose, glucose, and galactose.

With regards to Cherry cake product, it is clearly evident from Table (8) that the glucose and galactose content were significantly ($p \leq .05$) different between the control sample and other treatments. These differences might be due to different quantity of the added syrup. Data in table (8) also showed that, sucrose level was (7.55%) in the control sample, whereas it was (2.45%) in the 50% replacement level. The relatively high level of lactose (1.7%) in both control sample and 50% sugar replacement level might be due to the added cream (a major component of Cherry cake) that contains skim milk powder, as well as the residual amount of non hydrolyzed lactose that is already found in the added syrup. Many previous studies about the use of whey and whey products in cake products were reported.

Regarding Kunafah syrup product, the same trend for sugar composition can also be noticed. The sucrose level was (25.5%) in control sample compared to (10%) in the 50% sugar replacement level. The glucose level reached (7. 8%) in the 50% sugar replacement level compared to (6.1%) in the control syrup. Additionally, the 50% sugar replacement level contained (2.5%) fructose compared to (6.25%) in the control syrup. These determined amounts of both fructose and glucose were produced as a result of sucrose conversion under the effect of heat treatment during syrup preparations under the acid conditions. It was found that sucrose level showed pronounced decrease during storage of date jelly (Yousif *et el* ., 1990) . Whereas the glucose and fructose levels were significantly increased. These changes might be due to the sucrose inversion due to the acid environment (Yousif *et el* ., 1990). Finally, the determined amounts of galactose (5.5%) and lactose (1.65 %) in the 50% sugar replacement is due to the addition of the lactose hydrolyzed syrup that contained galactose and residual amount of non hydrolyzed lactose. On the other hand (0. 0%) of galactose and glucose were found in the control sample.

Table 8: sugar analysis of developed products

Product	Sucrose %	Glucose %	Galactose %	Fructose %	Lactose%
Syrup50%**	10.0000 A*	7.8000 A	5.5000 A	2.2500 A	1.6500 A
Control syrup	25.5000 B	6.1500 B	0.000 B	6.2500 B	0.000 B
Cake 50%	2.4500 A	2.8500 A	1.7000 A	0.000 A	1.7500 A
Cake control	7.5500 B	1.2500 B	0.000 B	0.000 A	1.4500 A
Bread 50%	1.9500 A	1.9000 A	0.9500 B	1.0000 A	0.3000 A
Bread control	2.1500 A	2.5500 A	0.000 A	2.6500 B	0.000 A

*Means in each column (for each developed product and the control) followed by the same letter are not significantly different at 95 % confidence

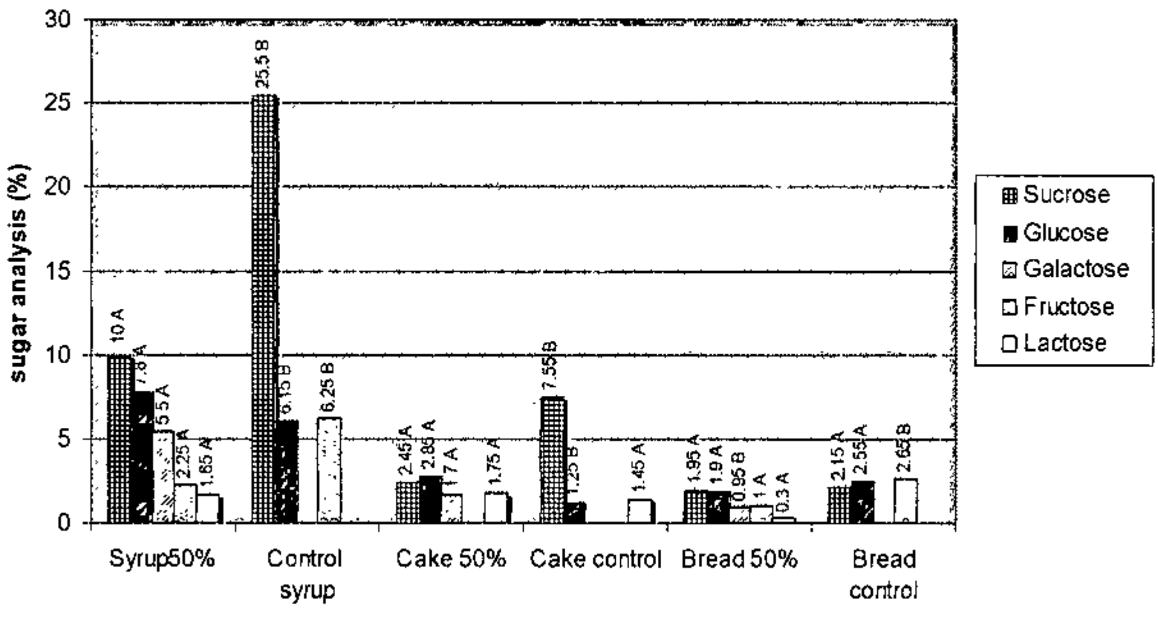


Figure 13 : Sugar analysis of developed products

4.6 Sensory evaluation results

Table (9) showed that there were significant differences ($p \leq 0.05$) between the new developed three products and the control samples. The sensory evaluation results of French-type bread showed that, bread prepared at 25% sugar replacement level was better than the control bread which achieved the best scores (2.46) compared to (3.00) of the control sample. But no significant differences were detected between the replacement level of 50 and 75% compared to the control sample since the 50% replacement sample achieved (2.70) scores, and the 75% replacement achieved (3.43) scores. These results were comparable to those obtained by Ogunrinola *et al.* (1988), who reported that, 22% of the panelist scored 'like very much" for the mineralized 75% hydrolyzed whey permeate syrups (HWPS) breads, 17% for the deminralized 50% HWPS bread, and 19% for control breads. In addition to that, the results indicated that there were slight significant preferable differences in proof time, bread quality among doughs formulated with sucrose (control) and HWPS.

With regards to the Cherry cake product, the sensory results revealed that there were no significant differences between the 25% replacement level treatment compared to the control sample. Whereas the control sample was significantly better than 50% and 75% replacement level. It can be also noted that, there were no significant differences between the two treatments 25 and 50% replacement level. Keshavarz and Nakai (1984) found that the sensory evaluation of the cake to which non fat dry milk was added to cake formula (rating 7.0) could be totally replaced by resin-lactase treated whey awarded (7.3) scores , which was significantly ($p \leq 0.01$) more acceptable than un treated whey cake rating (5.1) scores. Also there was no significant ($p \geq 0.05$) difference in acceptability between skim milk cake and resin- lactase treated whey cake.

For Kunafah product, it was noticed that there was no significant difference between the 25% replacement level and the control sample; the 25% treatment achieved (1.93) scores whereas the control sample a achieved (1.96) scores. On the other hand, it was noticed from the sensory evaluation results that, there were significant differences between the 50 and 75% replacement level compared with the control sample; the control sample achieved the best results (1.96) scores compared to (4.73) scores of both 50 and 75% replacement level. This result might be explained on the relative sweetness of added sugar. It is known that sucrose sugar has a high level of sweetness (100) compared to only (35) and (70-80) for the galactose and glucose respectively. However, most consumers favor the sweet taste especially for the confectionary products like (Kunafah and Cherry cake).

Studies on the relative sweetness of hydrolyzed lactose syrups have been reported by different researchers. Shah and Nickerson (1978) used a model system based on unflavored ice cream mix. The simulated hydrolyzed syrups were evaluated at two percent of substitutions for sucrose (25 and 50%) in the control mix, which contained 15% sucrose. The results showed a synergistic effect i.e, for example less sucrose was needed for equal sweetness when 25% of the sucrose was replaced with 70% hydrolyzed lactose syrup than when was replaced with 100% hydrolyzed syrup. Poutanen, *et al.* (1978) examined enzymatic conversion of glucose to fructose in glucose/ galactose syrups and found that the relative sweetness of the product was almost equal to that of sucrose. This approach may offer considerable potential to the dairy industry. There is also an application for a system that could be used for conversion of the comparatively low- sweetness galactose to either glucose or fructose. The specific enzyme should be found and its source should be developed commercially.

It would have a substantial effect on potential application of hydrolyzed products from lactose and on process economics (Zadow, 1984).

Table 9: sensory analysis of developed products

Product	25% replacement	50% replacement	75% replacement	Control	LSD values
French bread	2.4667 C*	2.7000 BC	3.4333 A	3.0000 AB	0.50093
Cherry cake	2.3667 BC	2.9333 B	4.5667 A	2.1000 C	0.57936
Kunafah	1.9333 B	4.7333 A	4.7333 A	1.9618 B	0.43285

*Means of duplicate

**Means in each row followed by the same letter are not significantly different at 95 % confidence

4.7. Bread storage study

The bread samples were evaluated organoleptically through 5 days of storage. Crakes were noted in the crust of the control sample compared with the treated samples (25, 50 and 75% sugar replacement level). This might be attributed to the evaporation of free water with storage, and that leads to shrinkage of the crust leading to formation of broken surface crust. This result might be ascribed to that the free water availability in the control sample was more than that of the treated sample. Additionally, such results might be due to the availability of more functional groups in the treated samples compared to the control. These functional groups like aldehyde, amines and carboxyl group will be bound with free water reducing the amount of free water in the treated sample compared to the control. Both moisture and weight loss (approximately 1%) during storage of bread samples at 30°C were observed (Pisesookbunterng *et al.*, 1983). The Inspection for visible microbial growth on bread samples was done daily through the unopened packages. No microbial growth was detected in all stored samples. These results might be explained based on the water activity, in which the level of water activity in both control and treated samples were extremely less than the minimal water activity required for any microbial growth which is (0.6) (Yousif *et al.*, 1990).

All stored samples were evaluated each day through the mentioned storage period. It was noted that all evaluated samples were accepted for the flavor. The bread was considered unacceptable if the bread showed visible signs of mould. Obvious changes in the bread color of the stored samples was not also recorded.

5. CONCLOUSIONS AND RECOMMENDATIONS

5.1 CONCLOUSIONS

1. Immobilization techniques used to immobilize β – galactosidase enzyme is a favorable method of whey lactose hydrolysis as long as there is possibility of reuse immobilized enzymes many times without without a significant decrease in its original activity. This satisfies the major objective of this research in which cost reduction of lactose conversion was a major target of our research.

2. There is possibility to produce sugar syrup from hydrolysis of whey lactose, since 85% of lactose hydrolysis could be obtained using fluidized bed reactor with optimization of hydrolysis parameters (pH, temperature, enzyme concentration, and flow rate). This syrup mostly contributes to nearly similar sweetness power as in sucrose sugar. Thus it is considered a successful alternative to sucrose and can be used as a sweetening agent in many food products.

3. Both protein and sugar content affected positively the shelf life of French-type bread product. Where the water activity of the treated samples (25, 50 and 75%) was less than the critical level of water activity required for microbial growth this result is clearly supported by storage study results.

4. Higher protein content in the treated samples will enhance the nutritional values of these products since whey protein is considered of a high quality type as well as increase the level of macro and micro minerals which might not be available in the original product

5. A clear correlation was noticed between sugar analysis results and the results of sensory evaluation. Sensory evaluation results were not the same for the three developed products compared to the control, corresponding to the differences in the level of sugar content. The new products incorporated with high level of sugar

replacement (50 and 75%) achieved low scores compared to the (25%) replacement level and the control.

6. Lactose level was very low in the three developed products. These results support one of the main objectives of this research to develop low or free lactose products for people who are suffering of lactose intolerance problem which might cover 70% of the world's population..

7. The (25%) sugar replacement level is the best treated product with regards to the sensory evaluation results and rheological properties study.

8. In Jordan since the (99%) of the whey which is mainly acid type whey is disposed in sewerage system causing an environmental problem, therefore there is a possibility to reduce the amount of whey disposed in sewerage system by utilization of the whey and whey components in many Jordanian food industries.

9. The results of the tests carried out on the newly developed products approved that it is possible to obtain a new product with almost the same or better than the control. Since no harmful effect on the quality was detected during this study, these results will enhance the idea of using whey lactose hydrolyzate in the formulating of food products.

5.2 Recommendations

1. It is recommended for the Jordanian dairy plants to develop a system for whey treatment to be used in different food industry. This objective can be achieved by encouraging the establishment of small lactose hydrolysis units in each dairy plant to benefit from the produced whey instead of disposing it and threatening the environment.

2. Utilization of acid whey hydrolysate in the local food processing, like bakery and confectionery industry, to enhance improving the technological and sensorial characteristics of food items (such as increased solubility, greater sweetening power and lower caloric content of the products (glucose and galactose have greater sweetening power than does lactose).

3. Further researches are needed to clarify many aspects with regards of using whey lactose hydrolysate in food industry like (candies, confectioneries, bakeries……etc) and to solve any defects that might appear during using this hydrolysate in the formulation of food products.

4. it is recommended to replace major part of Jordanian imports with regards to concentrated sugar syrups as glucose or fructose and dried whey by the sugar syrup produced locally from labneh whey; achieving of positive results from this research will encourage establishing of small lactose hydrolysis units in each dairy plant to benefit from the produced whey instead of disposing it and threatening the environment.

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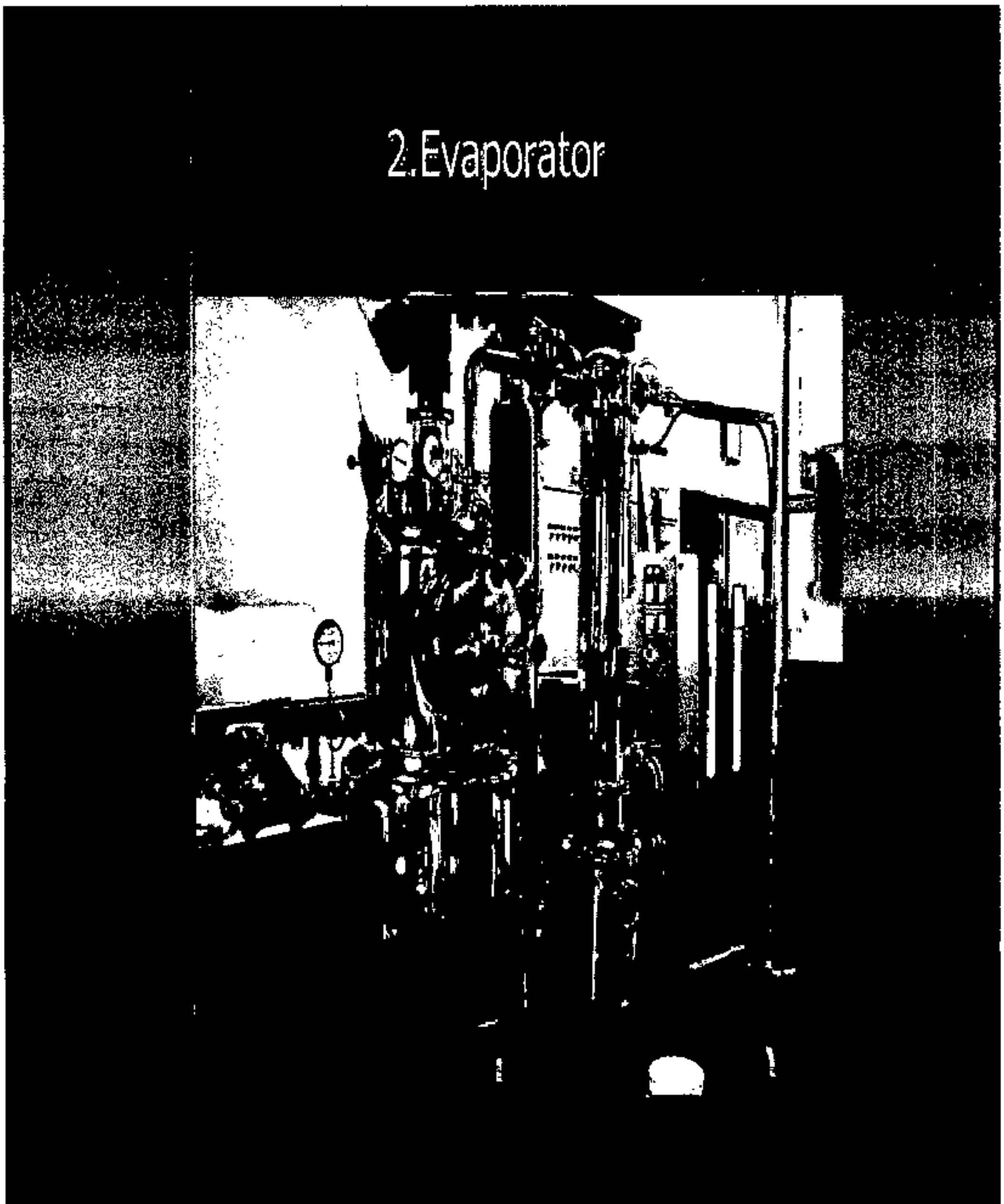
Zhou, Q. Z. and Chen, X. D. (2001), Immobilization of Beta-galactosidase on Graphite Surface by Glutaraldehyde, **Journal of Food Engineering**, 48,69-74.

APPENDICE

Appendix 1

Photos of the instruments used

- Evaporator instrument



APPENDIX 2
HPLC analysis sample

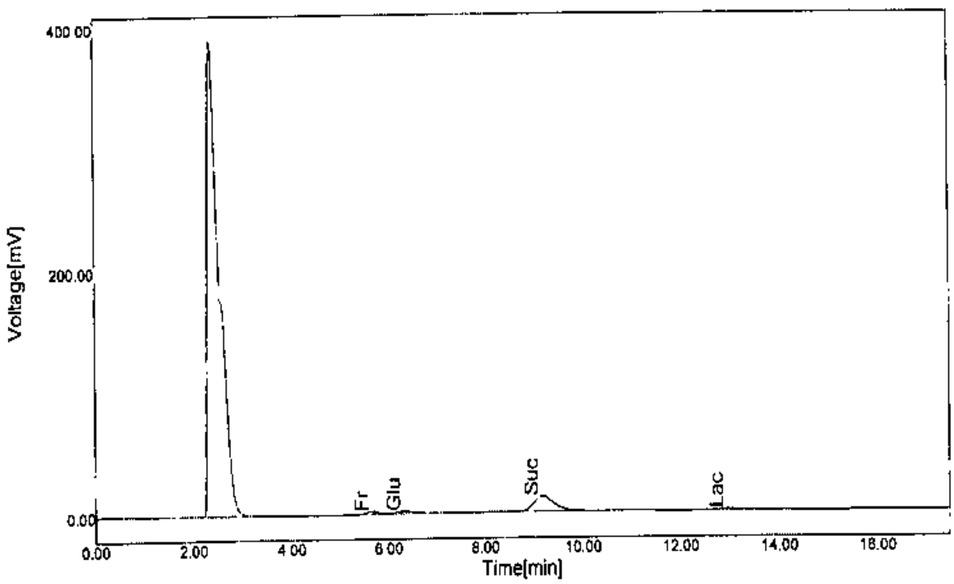
Analysis Report

Post:	Name:

Analysis

Sample Name: Cake cont. Sample ID: File:0210.RAW	Date: 2011-05-04 PM 12:28:43 Channel: 1. RI750F

Chromatogram

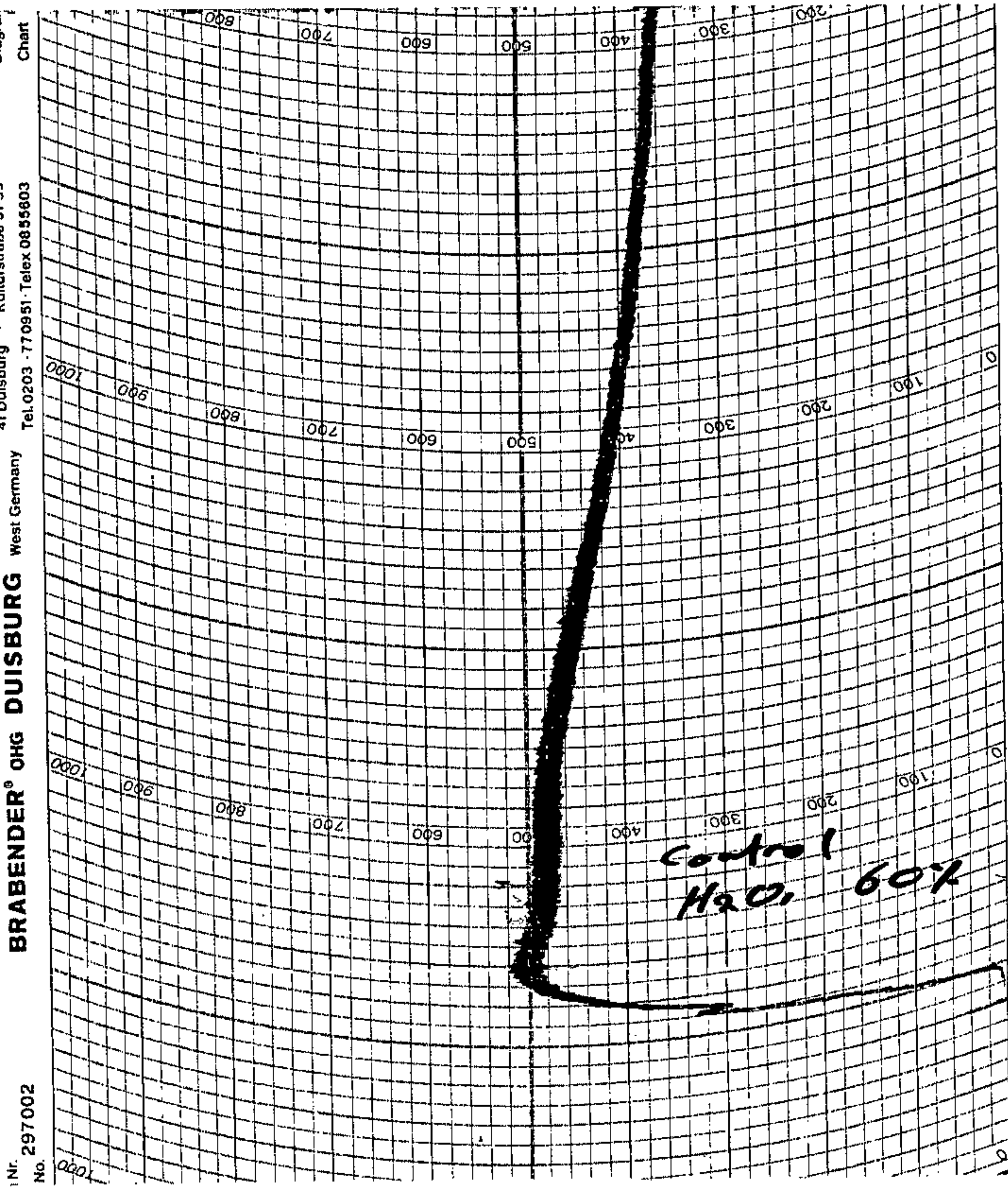


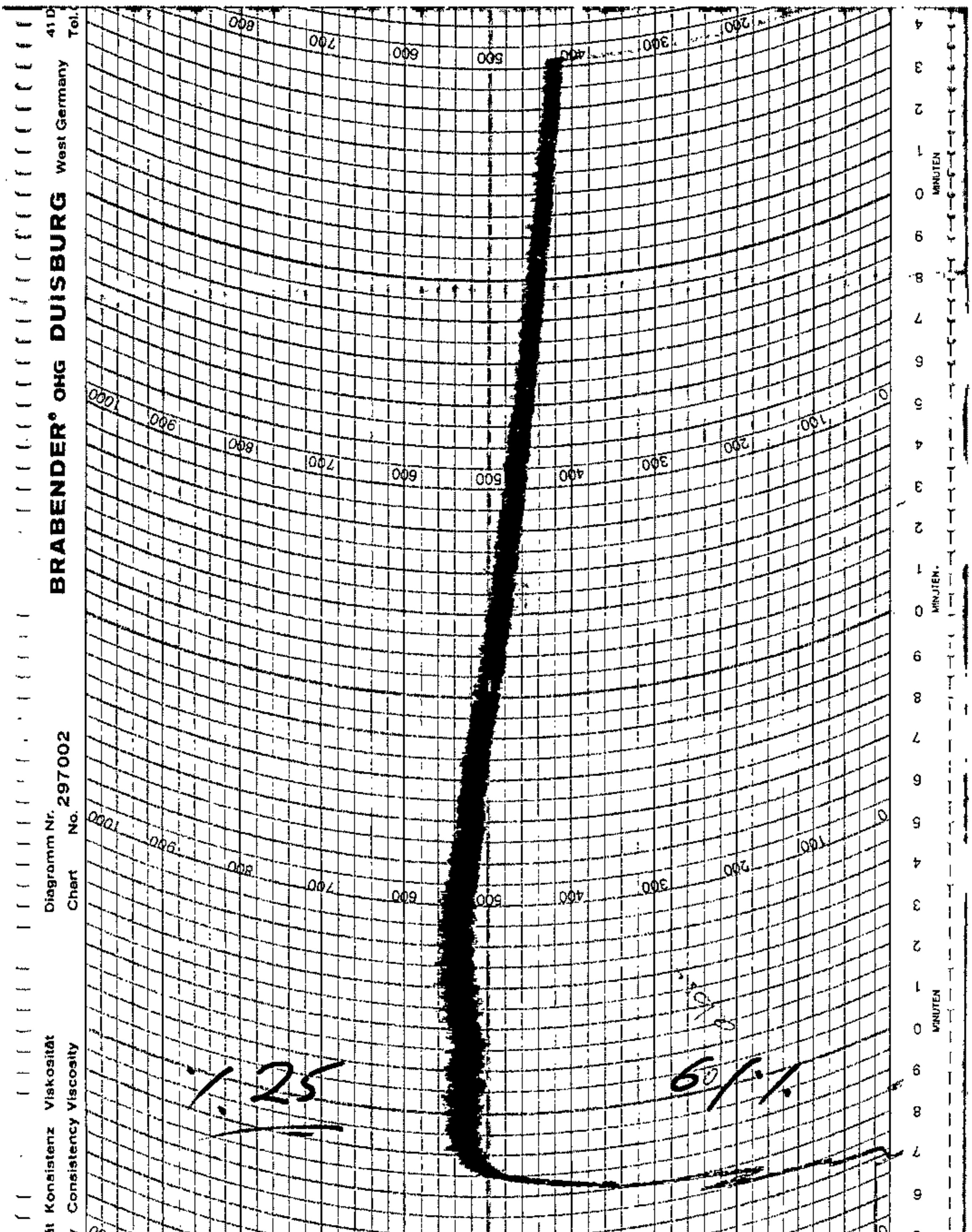
Result

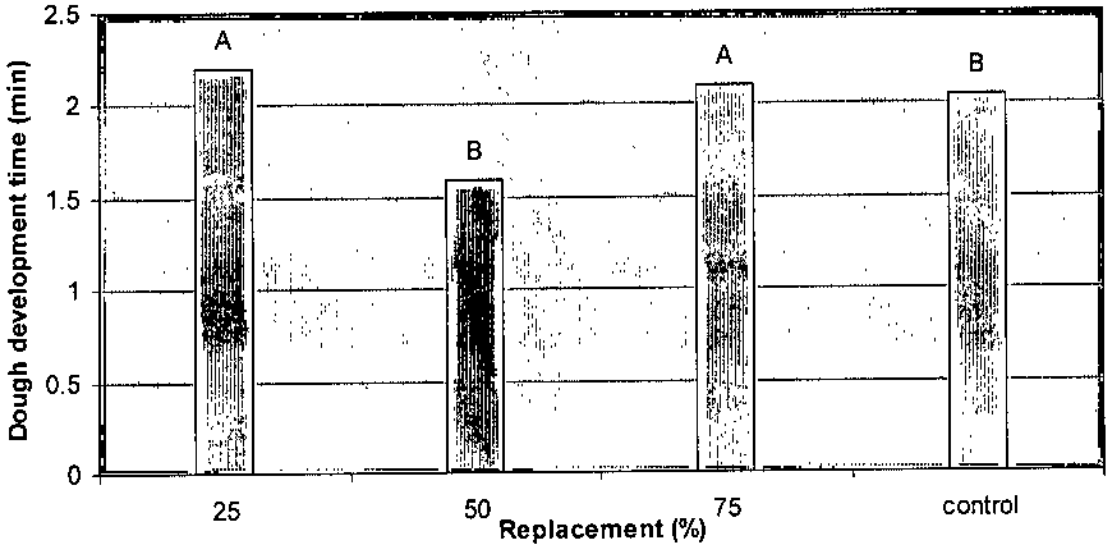
No.	Name	RT[min]	Area[mV*s]	Height[mV]	Amount[]	Area%
1	Fr	5.6667	55.7751	2.4613	0.0000	0.78
2	Glu	6.3167	69.4019	2.1468	0.0000	0.97
3	Suc	9.1667	399.0970	13.1471	0.0000	5.60
4	Lac	12.9500	55.1170	1.4352	0.0000	0.77
Sum			579.3909	19.1903	0.0000	

APPENDIX 3

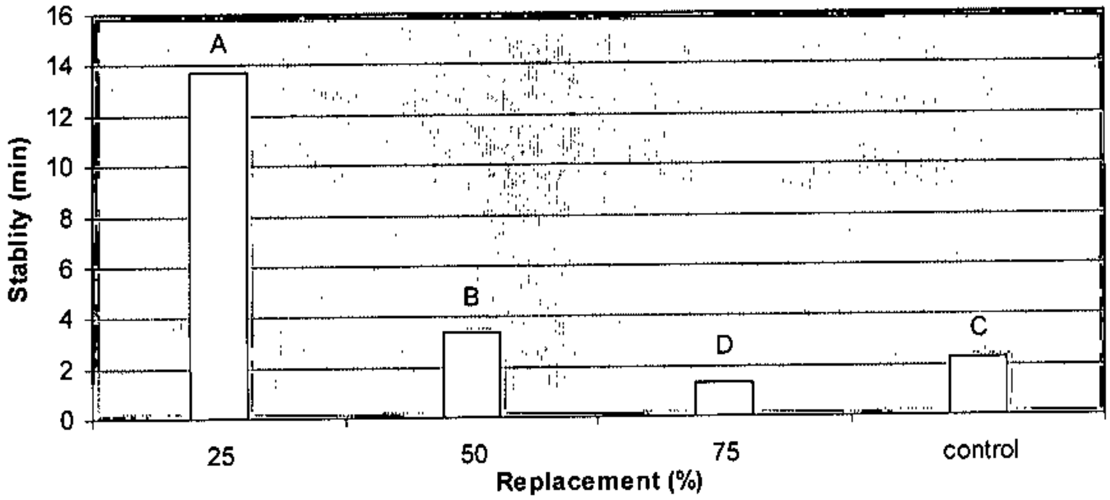
Farinagraph analysis (samples and bar charts)



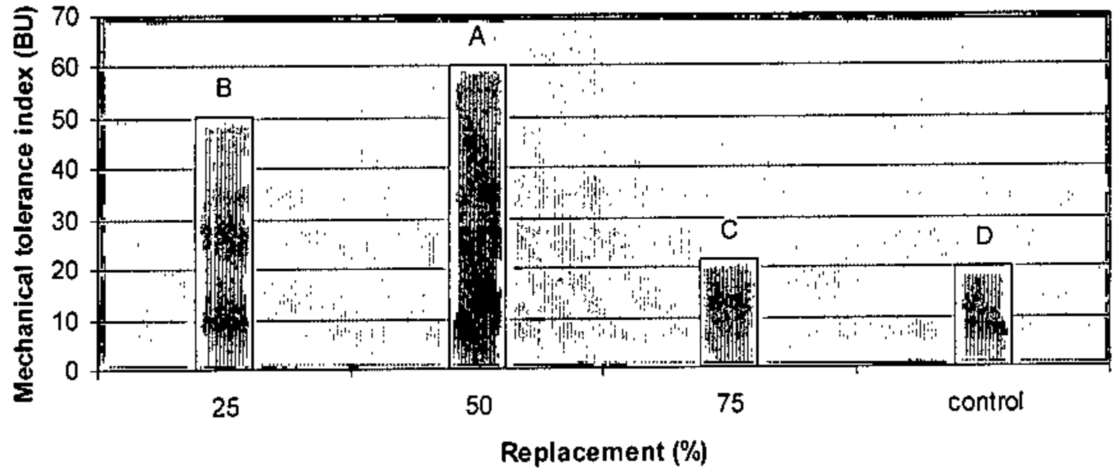

BRABENDER® OHG DUISBURG West Germany
Diagramm Nr.
Chart No. 297002
Konsistenz Viskosität
Consistency Viscosity
1.25
61%



Farinograph analysis (Dough development time)



Farinograph analysis (stabilty)



Farinograph analysis (Mechanical tolerance index)

APPENDIX 4

Statistical analysis sample

The SAS System

The Mixed Procedure

Product=bread (sensory evaluation)

Model Information	
Data Set	WORK.DABBOUR
Dependent Variable	result
Covariance Structure	Diagonal
Estimation Method	Type 3
Residual Variance Method	Factor
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
conc	4	25% 50% 75% control
Evaluator	15	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Dimensions	
Covariance Parameters	1
Columns in X	20
Columns in Z	0
Subjects	1
Max Obs Per Subject	120
Observations Used	120
Observations Not Used	0
Total Observations	120

Type 3 Analysis of Variance								
Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
Conc	3	15.666667	5.222222	Var(Residual) + Q(conc)	MS(Residual)	102	5.46	0.0016
evaluator	14	15.550000	1.110714	Var(Residual) + Q(evaluator)	MS(Residual)	102	1.16	0.3166
Residual	102	97.583333	0.956699	Var(Residual)	.	.	.	.

Covariance Parameter Estimates	
Cov Parm	Estimate
Residual	0.9567

Fit Statistics	
-2 Res Log Likelihood	325.0
AIC (smaller is better)	327.0
AICC (smaller is better)	327.0
BIC (smaller is better)	329.6

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Conc	3	102	5.46	0.0016
Evaluator	14	102	1.16	0.3166

Model Information	
Data Set	WORK.DABBOUR
Dependent Variable	result
Covariance Structure	Diagonal
Estimation Method	Type 3
Residual Variance Method	Factor
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	Levels	Values
conc	4	25% 50% 75% control
Evaluator	15	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Dimensions	
Covariance Parameters	1
Columns in X	20
Columns in Z	0
Subjects	1
Max Obs Per Subject	120
Observations Used	120
Observations Not Used	0
Total Observations	120

Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
Conc	3	110.091667	36.697222	Var(Residual) + Q(conc)	MS(Residual)	102	28.68	<.0001
evaluator	14	44.366667	3.169048	Var(Residual) + Q(evaluator)	MS(Residual)	102	2.48	0.0047
Residual	102	130.533333	1.279739	Var(Residual)	.	.	.	.

Covariance Parameter Estimates

Cov Parm	Estimate
Residual	1.2797

Fit Statistics	
-2 Res Log Likelihood	354.6
AIC (smaller is better)	356.6
AICC (smaller is better)	356.7
BIC (smaller is better)	359.3

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Conc	3	102	28.68	<.0001
Evaluator	14	102	2.48	0.0047

Model Information	
Data Set	WORK.DABBOUR
Dependent Variable	result
Covariance Structure	Diagonal
Estimation Method	Type 3
Residual Variance Method	Factor
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	**Levels**	**Values**
conc	4	25% 50% 75% control
Evaluator	15	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Dimensions	
Covariance Parameters	1
Columns in X	20
Columns in Z	0
Subjects	1
Max Obs Per Subject	119
Observations Used	119
Observations Not Used	0
Total Observations	119

Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term	Error DF	F Value	Pr > F
conc	3	230.657668	76.885889	Var(Residual) + Q(conc)	MS(Residual)	101	108.71	<.0001
evaluator	14	11.133900	0.795279	Var(Residual) + Q(evaluator)	MS(Residual)	101	1.12	0.3463
Residual	101	71.431618	0.707244	Var(Residual)	.	.	.	.

Covariance Parameter Estimates	
Cov Parm	**Estimate**
Residual	0.7072

Fit Statistics	
-2 Res Log Likelihood	291.5
AIC (smaller is better)	293.5
AICC (smaller is better)	293.5
BIC (smaller is better)	296.1

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Conc	3	101	108.71	<.0001
Evaluator	14	101	1.12	0.3463

Effect=conc Method=LSD(P<.05) Set=1

Obs	product	conc	evaluator	Estimate	Standard Error	Letter Group
1	bread	25%	_	2.4667	0.1786	C
2	bread	50%	_	2.7000	0.1786	BC
3	bread	75%	_	3.4333	0.1786	A
	bread	control	_	3.0000	0.1786	AB

Effect=evaluator Method=LSD(P<.05) Set=2

Obs	product	conc	evaluator	Estimate	Standard Error	Letter Group
5	bread		1	2.7500	0.3458	ABC
6	bread		2	3.0000	0.3458	ABC
7	bread		3	2.6250	0.3458	ABC
8	bread		4	2.6250	0.3458	ABC
9	bread		5	2.3750	0.3458	BC
10	bread		6	2.7500	0.3458	ABC
11	bread		7	3.5000	0.3458	A
12	bread		8	3.2500	0.3458	AB
13	bread		9	3.2500	0.3458	AB
14	bread		10	2.7500	0.3458	ABC
15	bread		11	2.8750	0.3458	ABC

Obs	product	conc	evaluator	Estimate	Standard Error	Letter Group
16	bread		12	3.1250	0.3458	ABC
17	bread		13	2.2500	0.3458	C
18	bread		14	3.5000	0.3458	A
19	bread		15	2.8750	0.3458	ABC

Effect=conc Method=LSD (P<.05) Set= LSD values for bread conc are 0.50093 (avg) 0.50093 (min) 0.50093 (max).
LSD values for bread evaluator are 0.97004 (avg) 0.97004 (min) 0.97004 (max).

APPENDIX 5

Questionnaire for the sensory evaluation of developed products

Questionnaire for Hedonic Scale

Name [illegible] DATE [illegible] PRODUT 7.4.2011

Taste these samples of Hamam Bread and check how much you like or dislike each one

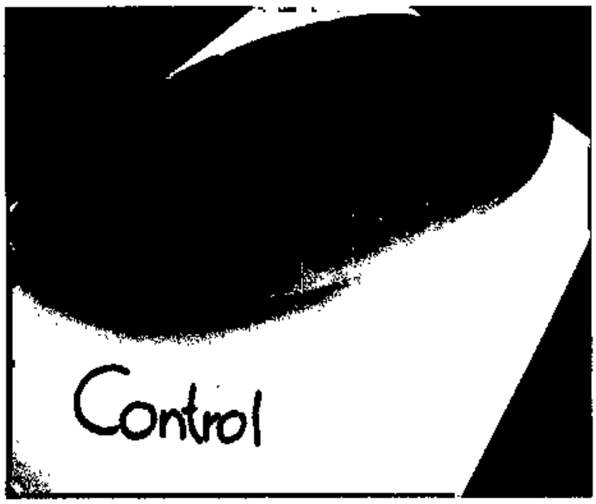
148	914	579	777
1. Like extremely	1. Like extremely	1. Like extremely	1. Like extremely
2. Like very much	(2) Like very much	2. Like very much	(2) Like very much
3. Like moderately	3. Like moderately	3. Like moderately	3. Like moderately
(4) Like slightly	4. Like slightly	(4) Like slightly	4. Like slightly
5. Neither like nor dislike	5. Neither like nor dislike	5. Neither like nor dislike	5. Neither like nor dislike
6. Dislike slightly	6. Dislike slightly	6. Dislike slightly	6. Dislike slightly
7. Dislike moderately	7. Dislike moderately	7. Dislike moderately	7. Dislike moderately
8. Dislike very much	8. Dislike very much	8. Dislike very much	8. Dislike very much
9. Dislike extremely	9. Dislike extremely	9. Dislike extremely	9. Dislike extremely

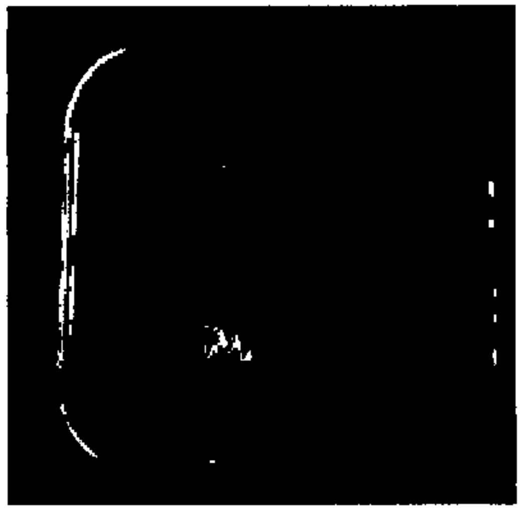
Comments

Appendix 6

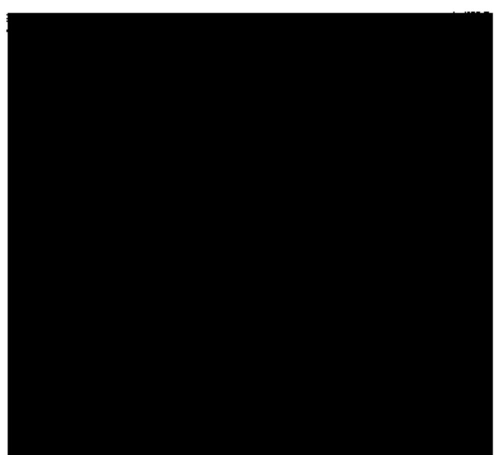
Photos of developed food product

1. French type bread

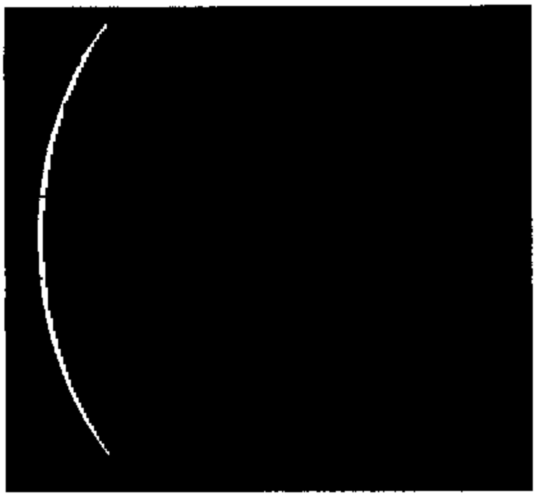


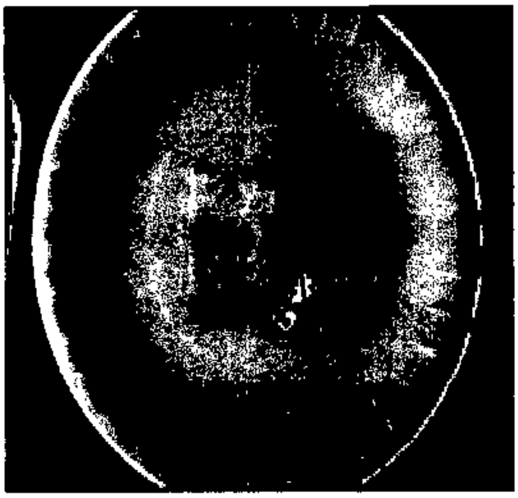


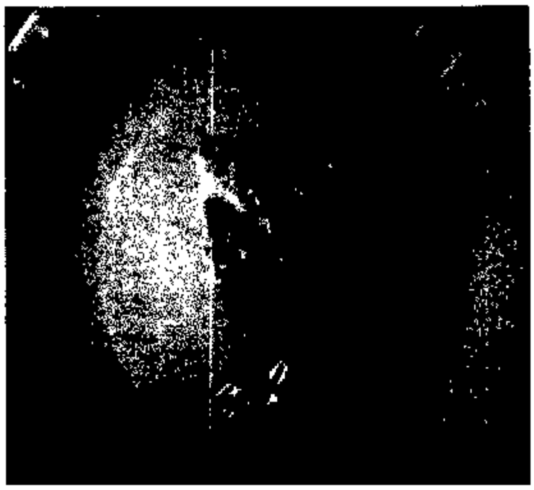


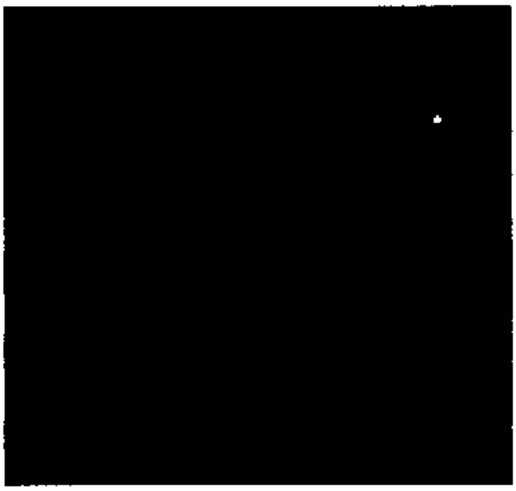


Cherry cake and Kunafah syrup











Kunafah syrup

تحلم لاكتوز شرش الحليب واستخدام (المحلول السكري) الناتج في صناعتي المخبوزات والحلويات

إعداد
وائل أحمد العمري

المشرف
الاستاذ الدكتور علي كامل الساعد

المشرف المشارك
الدكتور مالك صالح حدادين

ملخص

يعد تحلم لاكتوز شرش الحليب باستخدام أنزيم (بيتا – جالاكتوسايديز) من أحد أهم التقانات الحيوية المستخدمة في الصناعات الغذائية بسبب استخدامه في إنتاج منتجات ألبان منخفضة كمية اللاكتوز. تم جمع عينات شرش اللبنة الحامضي من مصنع الألبان في كلية الزراعة/الجامعة الأردنية . تم استخدام أنزيم (بيتا – جالاكتوسايديز) من خميرة (*Kluyveromces lactis*) في عملية التحلل عن طريق استخدام تقنية تثبيت الأنزيم (Immobilization) باستخدام مادة صوديوم ألجينيت، تم تثبيت الظروف المناسبة لعمل الأنزيم حيث تم الحصول على نسبة تحلل اللاكتوز بمقدار 85% وذلك باستخدام الظروف التالية (24%حجم/حجم لتركيز الأنزيم، 37 درجة مئوية ، 6.5 رقم هيدروجين ، و 10مل/دقيقة معدل الضخ للشرش) .

بعد الحصول على المحلول السكري تم استخدامه في إنتاج ثلاث منتجات (الخبز الفرنسي، الكيك، قطر الكنافة) حيث تم استبداله في السكر في هذه المنتجات بنسب (25 ، 50 و 75%) ، حيث لوحظ تحسن في الصفات الريولوجية لعجين الخبز من حيث زيادة الثباتية ونسبة امتصاص الماء عندما كانت نسبة الاستبدال (25%).

أظهرت نتائج التقييم الحسي أن نسبة الاستبدال (25%) أدت إلى تحسين المنتج وحصوله على درجات أعلى بالنسبة لمنتج الخبز وذلك من خلال التقييم الحسي ، بينما لم يكن هناك فروقات معنوية بين نسبة استبدال(25%) وعينة الشاهد بالنسبة لمنتجي الكيك والكنافة. أدى إضافة المحلول السكري إلى تحسين القيمة الغذائية لهذه المنتجات من حيث احتواء شرش الحليب على جودة عالية من البروتينات .